PERGAMON

biochemical systematics and ecology

Biochemical Systematics and Ecology 28 (2000) 457–470

www.elsevier.com/locate/biochemsyseco

Essential oil composition and variability of *Thymus lotocephalus* and *Thymus × mourae*

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Received 11 February 1999; accepted 9 June 1999

Abstract

The composition of the essential oils of four populations of *Thymus lotocephalus* G. López and R. Morales and one population of *T. × mourae* Paiva and Salgueiro, two endemic taxa from Portugal, was investigated mainly by GC and GC–MS. *T × mourae* is a natural hybrid between *T. lotocephalus* and *T. mastichina* (L.) L. subsp. *donyanae* R. Morales, which essential oil was analysed for the first time. In its oil, it was possible to find compounds of both parents, which could enable us to confirm its intermediate status between those two taxa. 1,8-Cineole and borneol were the main constituents in the essential oil of *T. × mourae*, whereas linalool, geranyl acetate and 1,8-cineol were the major ones in *T. lotocephalus*. Intermedeol was also an important constituent in the oils of both taxa. Nevertheless, the volatile oils of the four populations investigated of *T. lotocephalus* showed important differences among the main constituents. In order to study their infraspecific variability, the results obtained in the analysis of individual plants were submitted to a Principal Component and Chemometric Cluster Analyses. Five types of essential oils were found: linalool, 1,8-cineole, linalool/1,8-cineole, linalyl acetate/linalool and geranyl acetate. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: *Thymus lotocephalus*; *Thymus × mourae*; Lamiaceae; Essential oils; GC–MS; Infraspecific variability

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PII: S0305-1978(99)00088-5

1. Introduction

The chemical polymorphism of *Thymus* essential oils is a widespread phenomenon (Stahl-Biskup, 1991). Several chemotypes of several Portuguese *Thymus* sp. have been previously identified by us, such as: *T. zygis* Loefl. ex L. subsp. *sylvestris* (Proença da Cunha and Salgueiro, 1991), *T. zygis* Loefl. ex L. subsp. *zygis* (Salgueiro et al., 1993), *T. pulegioides* L. (Salgueiro et al., 1993), *T. capitellatus* Hoffmanns. and Link (Salgueiro, 1992), *T. carnosus* Boiss (Salgueiro et al., 1995), *T. villosus* L. subsp. *villosus* (Salgueiro et al., 1997a), *T. camphoratus* Hoffmanns. and Link (Salgueiro et al., 1997b), *T. mastichina* L. (Salgueiro et al., 1997c), and *T. albicans* Hoffmanns. and Link (Salgueiro et al., 1997c). Continuing our research on the composition of the essential oils of Portuguese thymes, we now report on the composition and variability of the essential oil of two endemic taxa, *T. lotocephalus* G. López and R. Morales and *T.* × *mourae* Paiva and Salgueiro.

T. × *mourae* is a hybrid between *T. lotochephalus* and *T. mastichina* (L.) L. subsp. *donyanae* R. Morales. It is a very rare plant occurring in a restricted area of the Algarve (Quinta de Marim), which we have described in 1994 (Paiva and Salgueiro, 1994). So, the composition of its essential oil is presented here for the first time.

T. lotocephalus is an endemic and also a rare species from SE Algarve. Little information about the essential oil of this taxon was available but its infraspecific variability was not known yet. Salgueiro (1992) reported a large amount of 1,8-cineole, camphor, linalool, linalyl acetate and α-pinene in the essential oil from a sample from Algarve, but more precise data were not given. Later, studies carried out by Figueiredo et al. (1993) on essential oils isolated from flowers, during full flowering, and from leaves, during the vegetative phase, of plants growing in the Botanical Garden of Lisbon showed marked differences in their composition. The composition of the oil isolated from flowers was dominated by linalyl acetate while in that one from leaves, 1,8-cineole was the major compound.

In the present work, we report the results of the qualitative and quantitative analyses of the essential oils of representative samples of four spontaneous populations of *T. lotocephalus* and one of *T.* × *mourae*, performed by GC and GC–mass spectrometry. Occasionally, analysis of the total essential oil by ^{13}C-NMR was also used to confirm the identity of the major volatile constituents. On the other hand, the oil of several individual plants of three populations of *T. lotocephalus* was also analysed mainly by GC and the results were submitted to Chemometric Cluster and Principal Components analyses, in order to study its infraspecific variability.

2. Material and methods

2.1. Plant material

Aerial parts of *T. lotocephalus* G. López and R. Morales and *T.* × *mourae* Paiva and Salgueiro (Lamiaceae) were collected at flowering stage, in May–June 1993, in SE Algarve. Four representative samples of *T. lotocephalus* from different localities were collected: Ludo (A), Amendoeira (B), Quinta de Marim (C) and Malhão-Amendoeira (D). *T.* × *mourae* grows only in Quinta de Marim (E), where one representative population sample was collected to perform the qualitative and quantitative analysis of the essential oil. Voucher specimens were deposited in the Herbarium of the Botanical Institute of the University of Coimbra (COI).

In order to study the infraspecific variability of the essential oils of *T. lotocephalus*, 37 individual plants from populations A–C were also collected at the same time as homogeneous samples of the corresponding populations. As *T.* × *mourae* is a very rare plant only three individual plants were available for this study.

2.2. Analysis of the essential oils

The essential oil yield of the air-dried plant material was determined according to the European Pharmacopoeia method (Conseil de l'Europe, 1983). Analysis of volatile oils obtained by hydrodistillation were carried out by GC and GC–MS on two fused-silica capillary columns with stationary phases of different polarities (Carboxaw 20M and methylsilicone SE-30), as previously described (Salgueiro et al., 1995; Adzet et al., 1989). ^{13}C-NMR spectra were recorded at 50 MHz, in $CDCl_3$, using TMS as internal standard.

Identification of components was made on the basis of their retention indices in relation to an homologous series of fatty acid Me esters and mass spectra, which were compared with those of our own library, literature data and authentic samples. When necessary, analysis of the total essential oil by ^{13}C-NMR was also used to confirm the identity of compounds with a percentage higher than 1%, as previously reported (Salgueiro et. al., 1995; Tomi et al., 1995).

The percentage composition of the essential oils was computed from the GC peak areas on the two columns, without using correction factors.

2.3. Infraspecific variability

The essential oil of each individual plant of *T. lotocephalus* was analysed by GC and, when necessary, by GC–MS and ^{13}C-NMR using the same analytical conditions indicated above. From all the volatile constituents, those which showed a percentage equal or higher than 2% were selected to be included in the multivariate analysis (Principal Component Analysis and Cluster Analysis) using PARVUS (Forina et al., 1988) and ESTATS (Tomàs et al., 1988) chemometric software packages, as previously reported (Salgueiro et al., 1995). Selected constituents (30) are shown in Table 1.

3. Results and discussion

The average yields of the essential oil of the air-dried aerial parts of the representative samples of *T. lotocephalus* and *T.* × *mourae* were 1.1 and 1% (v/w), respectively. Quantitative and qualitative analytical results are shown in Table 1, where the compounds are listed in order of their elution on a carbowax 20 M column. More

Table 1
Constituents of essential oils of Portuguese populations of *Thymus lotocephalus* and *Thymus × mourae*

Components[a]	% in essential oil of populations				
	T. lotocephalus				*T. × mourae*
	A	B	C	D	E
Tricyclene	0.1	0.2	0.2	0.2	0.1
α-Thuyene	0.2	0.4	0.3	0.5	0.1
α-Pinene[b]	6.0	5.8	5.0	9.0	2.2
Camphene[b]	1.9	0.8	1.5	1.3	3.9
β-Pinene[b]	1.0	0.5	0.7	0.7	1.4
Sabinene[b]	2.3	1.9	1.1	2.0	1.0
Myrcene	0.5	0.5	0.4	0.6	0.6
α-Phellandrene	t	t	t	—	t
α-Terpinene	0.1	0.2	0.1	0.2	0.1
Limonene[b]	1.2	1.2	1.2	2.0	0.4
1,8-Cineole[b]	12.2	11.8	6.9	12.7	23.5
cis-β-Ocimene	t	t	t	t	t
trans-β-Ocimene	t	t	t	t	t
γ-Terpinene	0.9	1.0	0.9	1.1	1.4
p-Cymene	1.3	1.2	0.5	0.9	0.7
Terpinolene	0.1	0.1	0.1	0.2	0.1
1-Octen-3-yl acetate	0.1	0.1	t	0.1	—
1-Octen-3-ol	t	t	t	t	—
Nonanal	t	—	t	t	—
Fenchone	0.1	0.1	0.1	t	—
Hexyl butyrate	0.1	t	t	t	t
Hexyl-2-methylbutyrate	t	t	t	t	t
cis-Linalool oxide	0.5	0.4	0.2	0.3	0.1
α-*p*-Dimethylstyrene	t	t	t	t	—
trans-Sabinene hydrate[b]	3.0	0.9	0.5	0.9	0.2
trans- Linalool oxide	0.2	0.2	0.1	0.1	0.1
Nerol oxide	0.1	t	t	t	—
Campholenal	0.3	0.2	0.1	0.1	t
β-Bourbonene	0.1	t	t	t	t
Camphor[b]	4.6	4.7	6.5	5.6	9.0
Linalool[b]	16.5	9.5	3.7	6.8	4.7
cis- Sabinene hydrate	0.2	0.1	0.1	0.2	0.2
α-Gurjunene	0.1	t	t	t	t
Linalyl acetate[b]	3.0	0.1	0.2	0.3	t
Pinocarvone	0.3	0.2	0.2	0.1	0.1
Bornyl formate	t	t	t	0.1	0.1
Bornyl acetate[b]	2.3	0.5	0.9	0.5	0.7
β-Elemene	t	t	—	t	—
trans-α-Bergamotene	t	t	t	t	—
Terpinen-4-ol[b]	2.8	3.9	0.9	2.1	1.4
β-Caryophyllene[b]	1.2	0.9	2.0	1.6	4.8
Myrtenal	0.3	0.4	0.3	0.1	—
allo-Aromadendrene[b]	0.3	1.5	1.0	1.7	0.5

Table 1—*continued*

Components[a]	% in essential oil of populations				
	T. lotocephalus				*T.* × *mourae*
	A	B	C	D	E
cis-Verbenol	0.7	1.2	0.8	1.2	0.2
trans-Pinocarveol	0.1	0.1	t	0.1	0.1
δ-Terpineol	0.7	0.9	0.6	1.1	1.2
trans-Verbenol[b]	2.0	3.1	1.9	3.3	0.6
α-Humulene	t	t	—	t	t
β-Cubebene	t	0.1	t	t	t
α-Terpineol[b]	1.7	1.8	0.8	1.2	2.2
α-Terpinyl acetate[b]	0.2	0.2	0.1	0.1	t
Borneol[b]	3.8	2.4	3.2	2.1	14.7
γ-Muurolene	t	t	—	t	—
Verbenone	0.6	0.7	0.4	0.5	0.2
Germacrene-D[b]	1.1	1.7	1.1	1.9	0.8
β-Bisabolene	t	0.2	0.2	0.3	t
Geranial	t	0.3	0.2	0.4	t
Bornyl isovalerate	t	t	—	t	—
Carvone	0.2	0.4	0.5	0.5	0.1
Bicyclogermacrene[b]	0.1	0.3	0.4	0.4	t
Decanol	—	—	—	—	t
Geranyl acetate[b]	0.2	5.8	15.0	3.5	0.5
Citronellol	—	—	—	—	0.3
δ-Cadinene	0.2	0.2	0.3	0.1	t
γ-Cadinene	t	t	0.1	t	t
Myrtenol	0.2	0.4	0.2	0.3	0.2
Cuminic aldehyde	t	—	—	t	—
Geranyl isobutyrate	—	0.1	0.1	0.1	t
Geranyl propionate	t	0.1	0.2	0.1	t
trans-Carveol	0.4	0.6	0.6	1.0	0.1
Cuparene	0.1	0.3	0.4	0.2	0.2
Geraniol[b]	0.1	0.2	0.6	0.3	t
p-Cymen-8-ol	0.4	0.9	0.3	0.4	0.2
Geranyl butyrate	t	0.2	0.4	0.1	0.1
Geranyl isovalerate	—	—	—	—	0.1
Geranyl isobutyrate	—	0.1	0.2	0.1	—
Isocaryophyllene oxide	0.6	0.3	0.4	0.2	0.3
β-Caryophyllene oxide[b]	5.9	3.5	3.6	3.8	3.5
Ledol[b]	1.4	3.0	2.9	3.4	1.9
Cubenol[b]	0.3	1.2	0.9	1.5	0.4
Globulol	0.4	1.0	1.0	1.0	0.1
Viridiflorol[b]	2.2	6.5	7.0	6.4	0.9
Cuminic alcohol	0.2	0.4	0.1	0.2	t
10-*epi*-γ-Eudesmol	—	t	t	t	—
Spathulenol[b]	1.2	1.1	1.0	1.0	0.2
Eugenol	t	t	—	t	—
T-Cadinol[b]	0.1	0.2	0.1	0.3	0.1

Table 1—*continued*

Components[a]	% in essential oil of populations				
	T. lotocephalus				*T.* × *mourae*
	A	B	C	D	E
10-*epi*-Cadinol	t	0.1	t	0.1	0.1
Thymol	t	t	—	—	—
α-Cadinol[b]	0.4	0.4	0.3	1.0	0.1
Intermedeol[b]	3.5	1.0	9.5	0.2	7.4
Eugenyl acetate	0.3	1.1	0.8	1.1	0.1
Monoterpene hydrocarbons	15.7	13.9	12.1	18.8	12.1
Oxygenated monoterpenes	58.1	53.0	47.0	46.6	61.1
Sesquiterpene hydrocarbons	3.4	5.4	5.7	6.5	6.6
Oxygenated sesquiterpenes	16.1	18.4	26.7	18.9	15.0
Others	0.6	1.3	0.9	1.4	0.3
Total identified	93.9	92.0	92.4	92.2	95.1

t: traces ⩽ 0.05%
[a]Components are listed in order of their elution from a Carbowax 20M column.
[b]Constituents selected for the multivariate analysis.

than 92% of the volatile oil was identified in each sample and in total 92 components were identified.

Oxygenated monoterpenes were shown to be the main group of constituents in all samples (46.6–61.1%). 1,8-Cineole was an important constituent in the essential oil of the four populations investigated from *T. lotocephalus* (6.9–12.7%). Nevertheless, important differences between other main components were found, particulary linalool (3.7–16.5%) and geranyl acetate (0.2–15.0%), which were the major compounds of populations A and C, respectively. These results showed some important differences between the populations, even between those from relatively close localities. All samples were also characterized by a high percentage of oxygenated sesquiterpenes (16.1–26.7%), some of which showing some variability, mainly intermedeol (0.2–9.5%). This compound was previously found by us in the essential oil of *T. camphoratus*, another endemic taxon from Portugal (Salgueiro et al., 1997b) and reported for the first time in Lamiaceae.

The main components of the oil of *T.* × *mourae* were 1,8-cineole (23.5%) and borneol (14.7%). Among sesquiterpenes, the oxygenated ones were detected in higher concentrations than the hydrocarbonated, being intermedeol (7.5%) the major one. As *T.* × *mourae* is a natural hybrid of *T. lotocephalus* and *T. mastichina* subsp. *donyanae*, with intermediate morphological features of its parental species, it was not surprising to find compounds of both parents in its essential oil. So, borneol comes from *T. mastichina* subsp. *donyanae* (Salgueiro et al., 1997c), 1,8-cineole from both parents and a high percentage of oxygenated sesquiterpenes, particulary intermedeol, from *T. lotocephalus*.

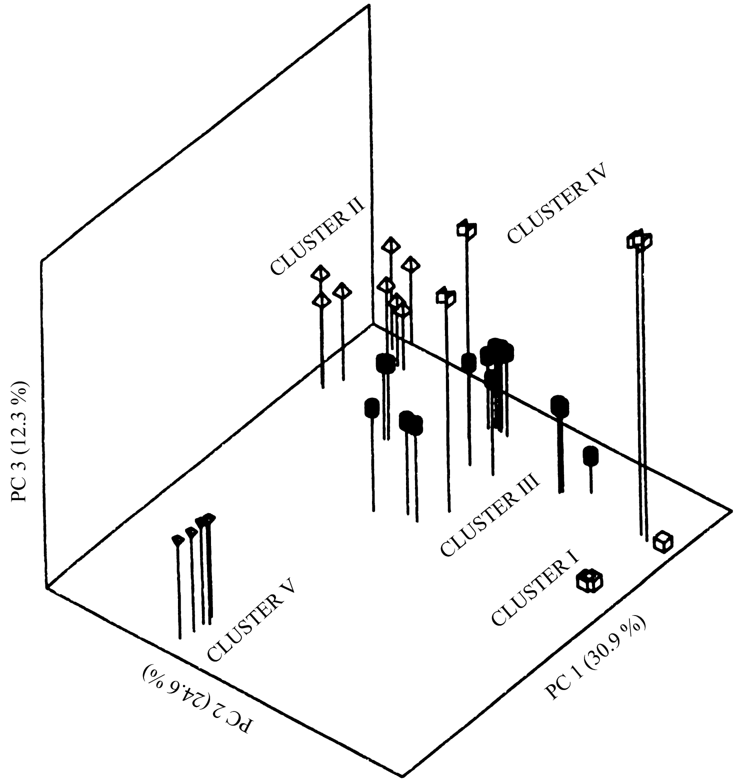

Fig. 1. Relative position of individuals of *T. lotocephalus* in the space defined by the first three principal components.

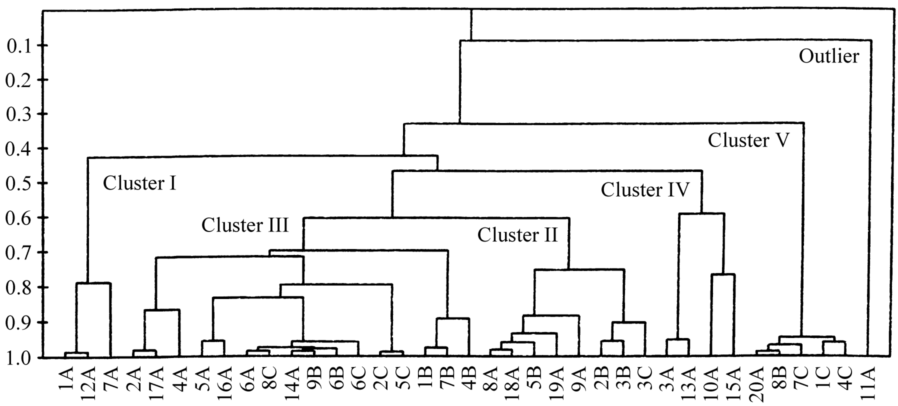

Fig. 2. Two-dimensional dendrogram obtained by Cluster Analysis of individual plants of *T. lotocephalus*. Horizontal: samples analysed (A, B and C indicate the population to which each sample belongs). Vertical: similarity levels between samples.

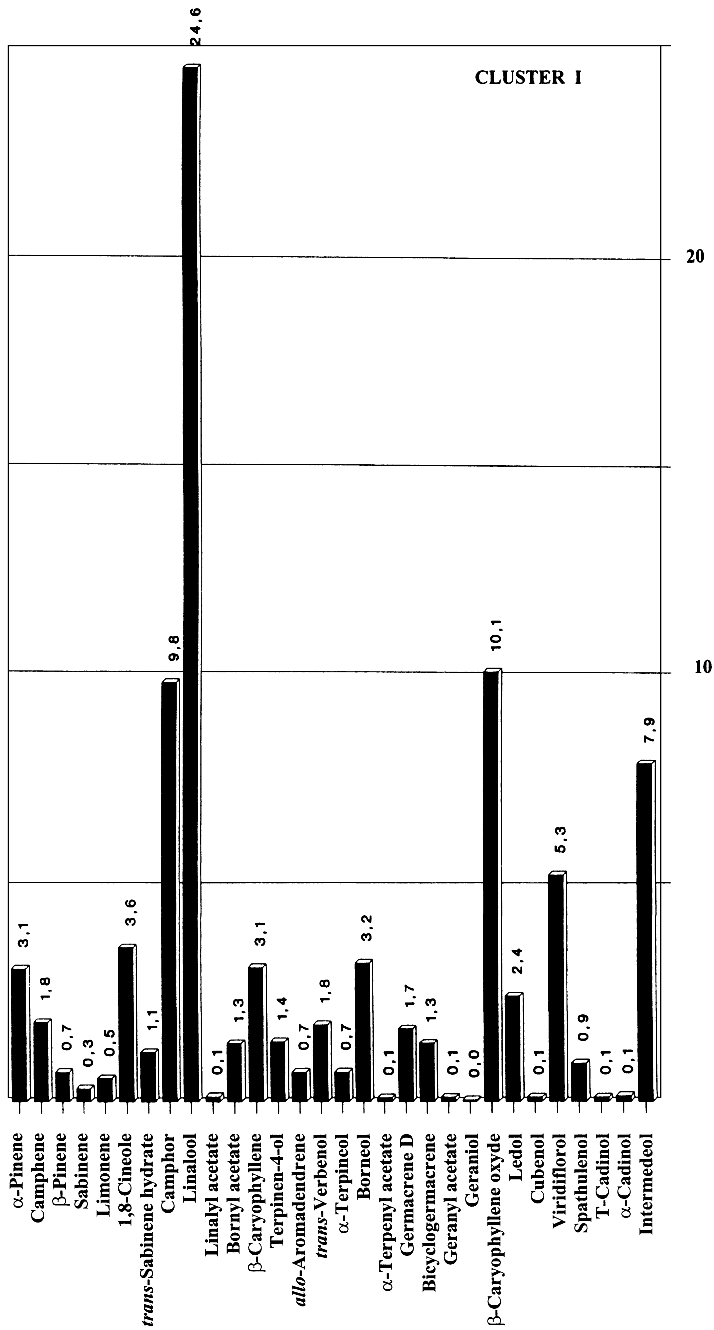

Fig. 3. Mean chemical composition of essential oil of cluster I. Vertical: mean percentage of essential oil.

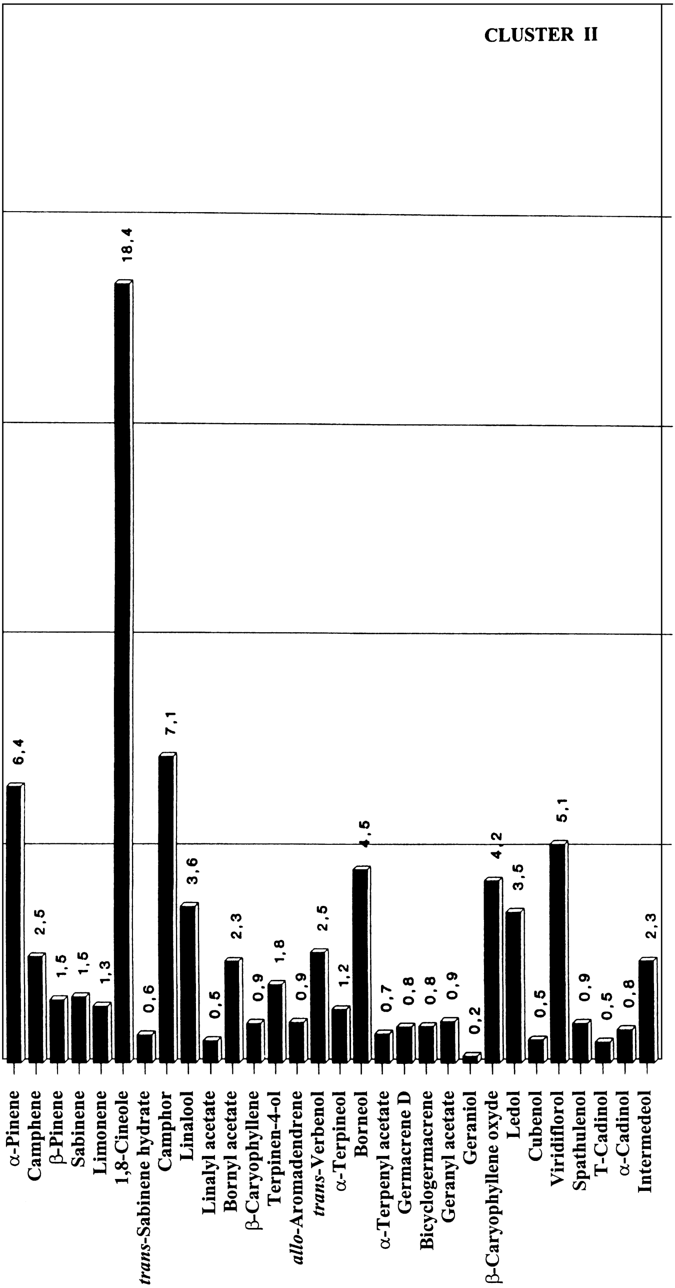

Fig. 4. Mean chemical composition of essential oil of cluster II. Vertical: mean percentage of essential oil.

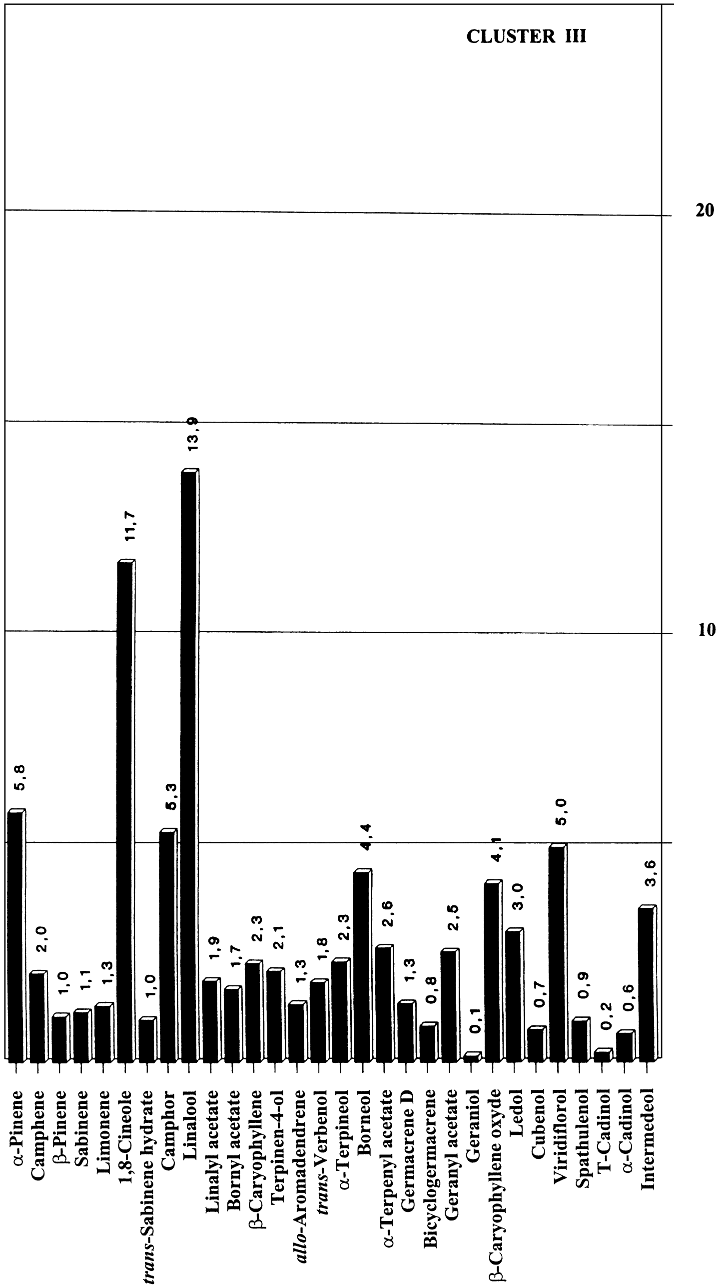

Fig. 5. Mean chemical composition of essential oil of cluster III. Vertical: mean percentage of essential oil.

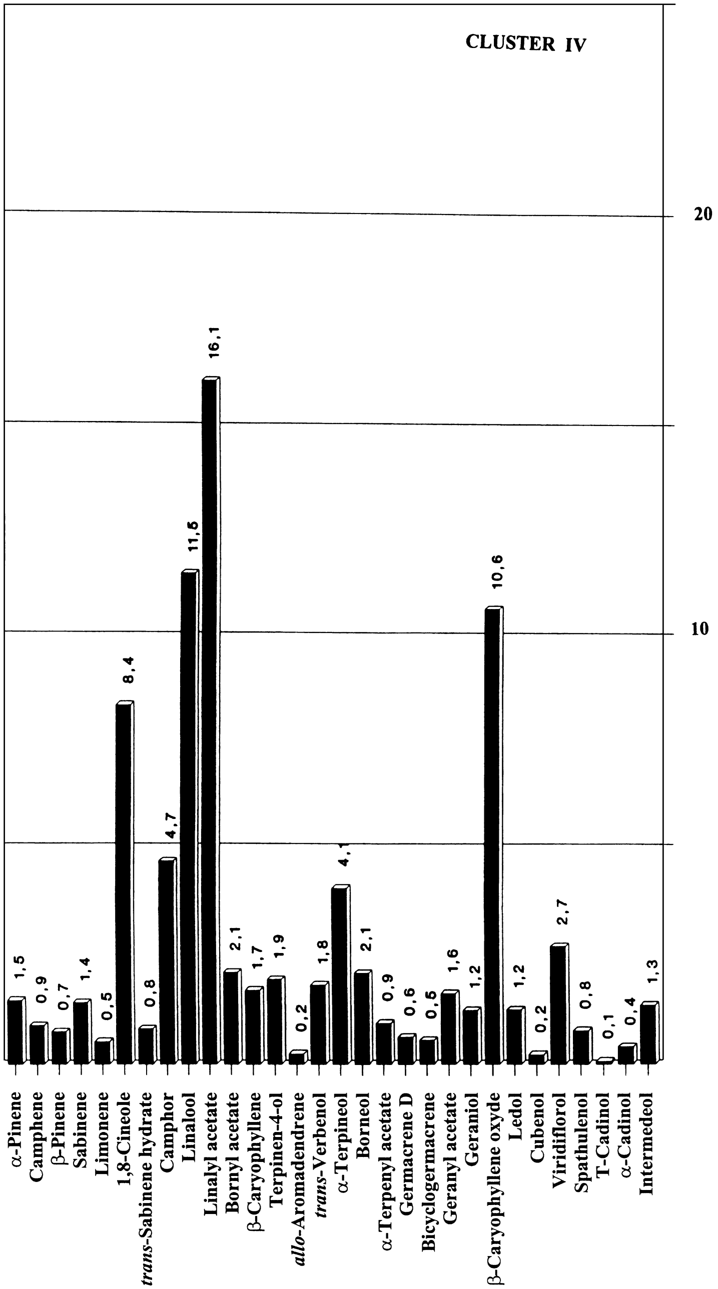

Fig. 6. Mean chemical composition of essential oil of cluster IV. Vertical: mean percentage of essential oil.

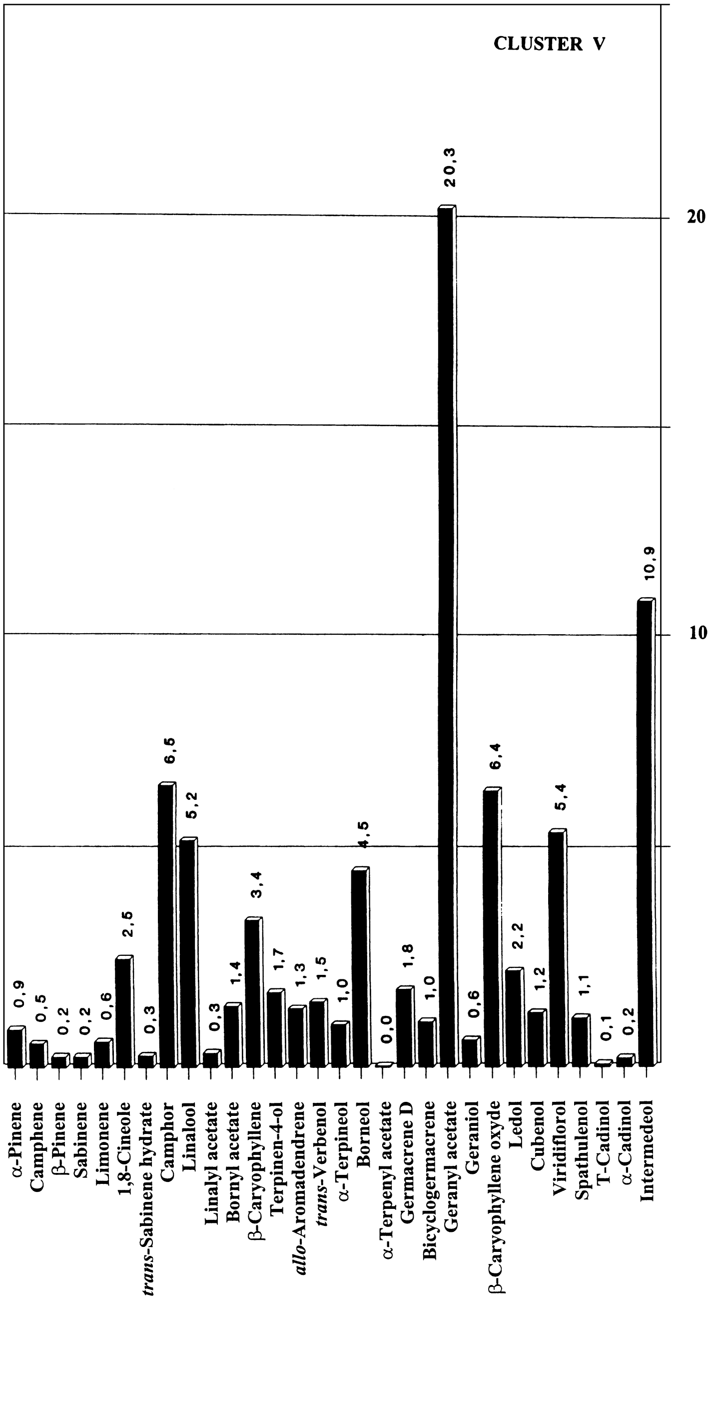

Fig. 7. Mean chemical composition of essential oil of cluster V. Vertical: mean percentage of essential oil.

Table 2
Percentages of individuals of each locality belonging to each cluster

		Cluster				
Population	N	I	II	III	IV	V
A	20	15.8	21.1	36.8	21.1	5.2
B	9	—	33.3	55.6	—	11.1
C	8	—	12.5	50.0	—	37.5

The application of multivariate analysis to the results obtained of the volatile oils from individual plants of *T. lotocephalus* allow us to establish five different types of essential oils, which were differentiated by both the Cluster Analysis and PCA (Figs. 1 and 2, respectively): Cluster I, characterized by a high content of linalool (8.3% of the samples analysed); Cluster II, in which 1,8-cineole was the main constituent (22.2% of the samples analysed); Cluster III, which shows an intermediate composition between clusters I and II, its main constituents being linalool and 1,8-cineole (44.5% of the samples analysed); Cluster IV, characterized by a high content of linalyl acetate and linalool (11.1% of the samples analysed); Cluster V, with substantial percentages of geranyl acetate (13.9% of the samples analysed). In the essential oils of Cluster I, linalool was the major constituent while linalyl acetate attained only 0.2%. On the other hand, in Cluster IV, linalyl acetate was the main compound, followed by linalool. These two clusters showed, among the sesquiterpenes, a high concentration of β-caryophyllene oxide. Meanwhile, intermedeol was abundant only in Cluster I. This compound was also important in Cluster V. The average percentage composition of each cluster is showed in Figs. 3–7.

Table 2 shows the percentages of essential oil of each type found in every locality. These results showed a high infraspecific variability within the essential oils from plants collected in the same stage of development in spite of the small size of the populations. Even so, we found the five types of essential oils in the same population, as, for instance, in population A. This fact indicates that the chemical polymorphism of the essential oil of *T. lotocephalus* might be due, in part, to genetic characters.

The essential oils of the individual plants of *T.* × *mourae* were characterized by their 1,8-cineole and borneol high content, which is in accordance with the results found in the analysis of the representative sample of this population. As we have studied only three individual samples it was not possible to conclude whether this taxon is or not chemopolymorphic. More plant material is necessary, but in order to protect the small and rare population of this hybrid, we did not collect large amount of plants. Nevertheless, we are look forward to find more populations for further research.

Acknowledgements

The authors are grateful to Professor Dr. Joseph Casanova and to Dr. Felix Tomi (University of Corsica) for providing ^{13}C-NMR spectra.

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