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VARIABILITY OF THE ESSENTIAL OIL OF *THYMUS PIPERELLA*

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Key Word Index—*Thymus piperella*; Lamiaceae; pebrella; essential oil; chemotypes; multivariate analysis.

Abstract—Chemometric investigation of the infraspecific variability of the essential oil of 31 populations of *Thymus piperella* led to the differentiation of three main chemotypes; type A: *p*-cymene-carvacrol-γ-terpinene (11 populations); type B: *p*-cymene-thymol (5 populations); type C: *p*-cymene-carvacrol (15 populations). The compound *p*-cymene is the most abundant constituent in all samples. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Essential oil chemotypes have been reported in *Thymus vulgaris* subsp. *vulgaris* [1], *T. vulgaris* subsp. *aestivus* [2], *T. mastichina* [3], *T. villosus* subsp. *lusitanicus* [4], *T. nitens* [5], *T. herba-barona* [6], *T. praecox* subsp. *arcticus* [7, 8] and *T. longicaulis* and *T. willkomii* [9]. The origin of such races or varieties is tied in with isolation phenomena or with ecological characteristics. Environmental changes, such as mineral status of substrate and climatic characteristics, have well-established effects on composition and variation of the essential oils [10, 11].

Thymus piperella L. is an endemic Iberian eastern taxon, extending over approximately 8000 km^2 in the province of Valencia. The morphological characteristics, as well as the palynological [12], cariological [13] and chemical [12–17] contributions show evident taxonomic independence from neighbouring species within the genus; it is placed on its own in the section *Piperellae* Willk. To date no investigations have been reported on the variability of the *T. piperella* essential oils, in spite of its different phytosociological affinities [18]. In the present work, the composition and chemical variability of essential oil of this species is carried out, showing chemotypes and geographical distribution.

RESULTS AND DISCUSSION

The maximum yield in essential oils is produced in the period of maximum flowering (August and September). Essential oil yield from the leaves and flowers was 1.8% (v/w, S.D. = 0.4) and for the flowering stems, it was 0.8% (v/w, S.D. = 0.3). The qualitative results from the samples studied indicated *p*-cymene (47.8%), carvacrol (14.6%), γ-terpinene (7.7%) and thymol (4.9%) as the major components of the essential oils; these compounds are related biosynthetically. The epoxide of caryophyllene is described for the first time in the essential oil of *T. piperella*, representing approximately 1% (Table 1).

A data matrix from the principal components of the essential oil detected in 31 samples is elaborated. Prior to the PCA (principal components analysis) and CLUSTER analysis, the variables were standardised for a normalised PCA. The set of data is processed through the MVSP statistical package for chemometric purposes and by the NTSYS package for the calculation and elaboration of the CLUSTER through the SAHN method (Sequential, Agglomerative, Hierarchical and Nested) from a data matrix of similarity values. Euclidean distance was selected as a measure of similarity between samples and averages linkage method, weighted pair group was used as agglomerative algorithm. The application of this multivariate analysis for the clustering process has provided a consistent classification of the *T. piperella* oils. It revealed the presence of three groups, which have been easily related to the levels of *p*-cymene, carvacrol, γ-terpinene, and thymol. The chemotype *p*-cymene–carvacrol–γ-terpinene is characterised by the combination of the *p*-cymene (43%, S.D. = 9.2), carvacrol (15.8%, S.D. = 5.5) and the γ-terpinene (14.0%, S.D. = 4.4). The chemotype *p*-cymene–thymol con-

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Table 1. Principal components of the essential oil of *T. piperella* and characterisation of chemotypes

Components	Chemotype □ (n = 11)		Chemotype ● (n = 5)		Chemotype △ (n = 15)	
	Mean (%)	S.D.	Mean (%)	S.D.	Mean (%)	S.D.
Unidentified	1.07	0.47	1.16	0.15	0.74	0.35
α-pinene	2.64	1.15	2.10	0.80	1.78	1.20
Camphene	0.89	0.36	1.26	0.42	1.30	0.81
β-pinene	0.42	0.35	0.32	0.36	0.49	0.29
Myrcene	1.33	0.45	1.36	0.57	0.79	0.45
p-cymene	43.27	9.14	44.82	10.64	52.10	16.22
Limonene	3.15	1.74	1.66	0.87	1.86	1.14
1.8-cineole	1.25	0.99	0.60	0.52	1.37	1.40
γ-terpinene	13.98	4.41	4.34	3.99	4.29	3.62
Linalol	2.88	2.04	1.40	1.50	2.40	1.24
Camphor	0.02	0.06	0.04	0.09	0.34	0.75
Borneol	1.84	0.67	2.54	0.87	2.80	1.65
4-terpineol	0.45	0.24	0.52	0.19	0.65	0.24
α-terpineol	0.10	0.11	0.12	0.08	0.25	0.23
Unidentified	0.79	0.87	3.84	1.65	0.93	1.29
Thymol	1.51	2.75	22.96	8.98	1.28	1.22
Carvacrol	15.76	5.48	1.70	0.91	18.10	7.62
β-caryophyllene	3.10	0.98	2.22	0.72	1.54	0.89
Caryophyllene oxide*	0.92	0.45	1.26	0.90	0.89	0.55
Unidentified	2.01	1.47	2.46	2.07	3.35	3.70

Chemotypes: □ *p*-cymene carvacrol γ-terpinene, ● *p*-cymene thymol, △ *p*-cymene carvacrol.
* Compound not detected previously in essential oils of *T. piperella*.

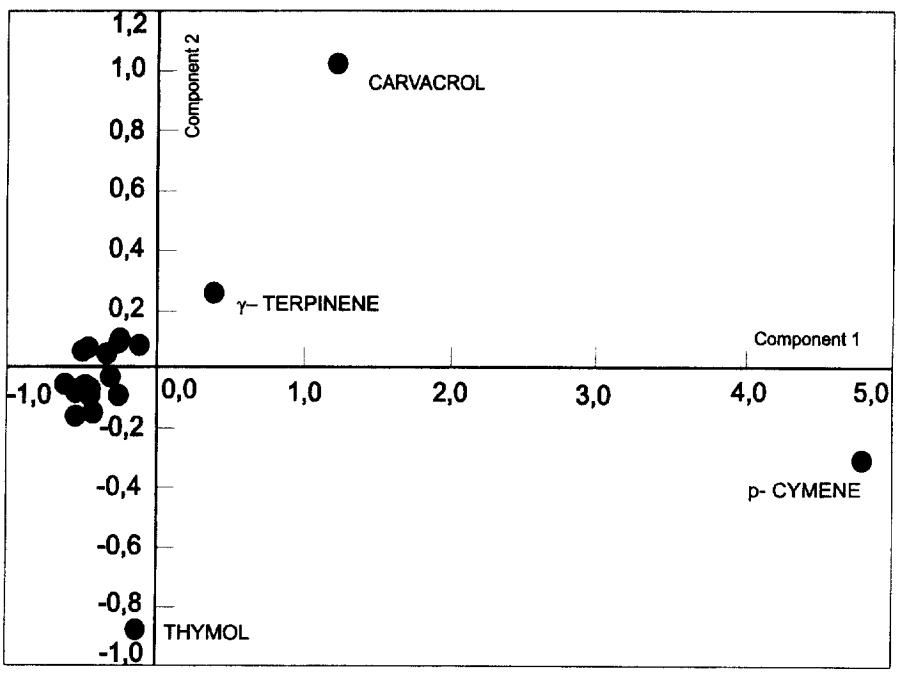

Fig. 2. Relative position of the essential oil constituents with the two first principal components (94.6% of total variance explained).

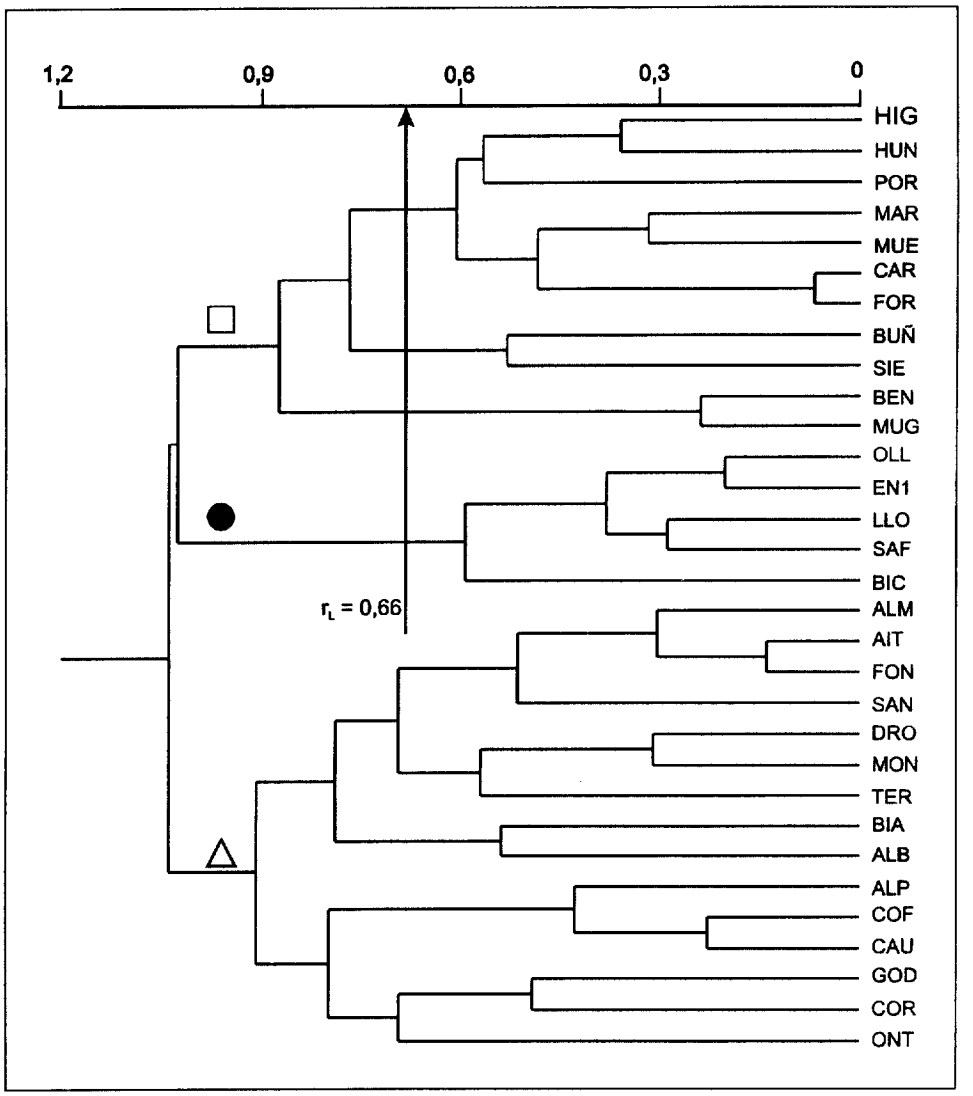

Fig. 1. Dendrogram obtained from the cluster analysis. Vertical scale: geographic origin of samples analysed (essential oils from 25 samples/population). Horizontal: differentiation level between samples. □, Chemotype *p*-cymene carvacrol γ-terpinene; ●, chemotype *p*-cymene thymol; △, chemotype *p*-cymene carvacrol. r_L = Minimum value required to accept differentiated groups at level $P \leqslant 0.05$.

tains thymol (23.0%, S.D. = 9.0) together with *p*-cymene (44.8%, S.D. = 10.6). The chemotype *p*-cymene–carvacrol is characterized by *p*-cymene (52.1%, S.D. = 16.2) and carvacrol (18.1%, S.D. = 7.6). One must emphasise the high percentage of *p*-cymene (87.7%) that can be reached in this chemotype.

The principal component analysis established the combination of variables, essential oil constituents or samples (localities), that account for the largest amount of variance to form the first principle component. In Fig. 2, axis 1 represents a high relative contribution to explain the variability in the set of data (88.4% of total). *p*-Cymene, the principal constituent, is strongly related to the first component. On the other hand carvacrol and thymol are also related to the second component (6.14% of total). γ-Terpinene, a compound characteristic of the chemotype is not related to the other two components present.

Discriminant analysis (DA) has been performed to determine a test of validity of the discriminant function, which can help predict chemotypes, based on the values of the determinate quantitative variables. The 31 samples were used to develop a model to discriminate among the three levels of proposed chemotypes. Three predictor variables, carvacrol, γ-terpinene and thymol, were entered. The two discriminating function with *P*-values less than 0.05 are statistically significant at the 95% confidence level (Table 2, B).

Table 2. Discriminant analysis summary of *T. piperella* chemotypes. Number of samples: 31; independent variables: 3 (carvacrol, γ-terpinene and thymol)

A—Discriminant functions	Eigenvalue	Relative percentage	Canonical correlation	
F1	7.14	84.31	0.93	
F2	1.33	15.69	0.75	
B—Functions derived	**Wilks lambda**	**Chi-square**	***DF***	***P*-value**
F1	0.05	79.47	6	0.000
F2	0.42	22.83	2	0.000
C—Standardized coefficients of discriminant functions		**Carvacrol**	**γ-Terpinene**	**Thymol**
F1		−0.43	−0.44	0.96
F2		−0.33	0.93	0.11

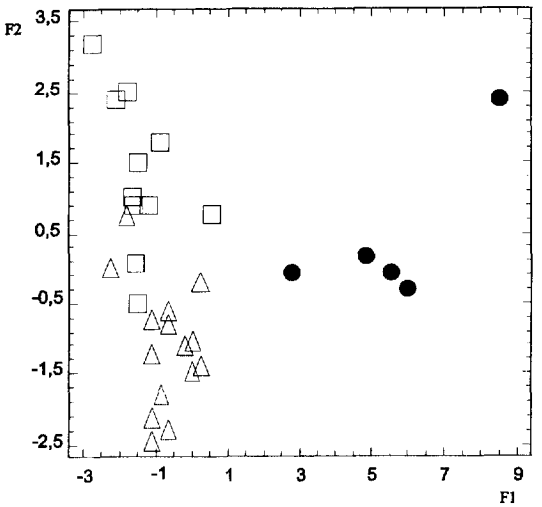

Fig. 3. DA-scatterplot of 31 samples of *T. piperella*. F1: discriminant function 1; F2: discriminant function 2. : □, Chemotype *p*-cymene carvacrol γ-terpinene, ●, chemotype *p*-cymene thymol, △, chemotype *p*-cymene carvacrol.

The *p*-cymene–thymol chemotype shows a very high correspondence with the discriminant function 1 (F1, Fig. 3) because of positive marked correlation of the thymol variable (Table 2, C). The *p*-cymene–carvacrol and *p*-cymene–carvacrol–γ-terpinene chemotypes are more related with the second discriminant function (F2) where the contribution of the variable thymol is less evident than carvacrol and γ-terpinene. The former contributes to the negative and positive values of this function respectively.

In the geographic distribution (Fig. 4), partial overlapping of the areas happen for the *p*-cymene–carvacrol and *p*-cymene–carvacrol–γ-terpinene chemotypes which represent the great part of the area of dispersion of the species. Chemotype *p*-cymene–thymol is best defined and occupies a smaller area.

EXPERIMENTAL

Plant material

The samples obtained from 31 populations (25 plants/population) are representative of the different dispersion areas needed in order to study the variability intraspecific of the essential oil. The localities are in the province of Valencia: Bicorp (BIC), Buñol (BUN), Caroche (CAR), Caudete (CAU), Cofrentes (COF), Cortes (COR), La Drova (DRO), Enguera (EN1), Fontanares (FON), Forestales (FOR), Godelleta (GOD), Higueruelas (HIG), Llosa (LLO), Mongente (MON), Muela (MUE), Ollería (OLL), Ontenient (ONT), Portera (POR), La Safor (SAF), Santis (SAN), Siete Aguas (SIE), Teresa (TER). In province of Alicante: Aitana (AIT), Benifallim (BEN), Biar (BIA), Mariola (MAR). In Albacete: Albatera (ALB), Almansa (ALM), Alpera (ALP), La Hunde (BUN), and Mugrón (MUG).

They were dried in air, being separated from the inflorescences and the leaves from the stems.

Chemical method

Essential oil was obtained by hydrodistillation using a Clevenger's glass apparatus being obtained data from the yield for each population. The qualitative analysis was carried out with a GC-MS using a capillary column BP*5 non polar (25 m × 0.22 i.d.) on fused silica (film thickness 0.25 microm) and with the temperature programmed in two stages: $T_1 = 60$°, rate 1 = 5°/min; $T_2 = 170$°, rate 2 = 10°/min; $T_3 = 280$°. The bearing gas was helium. The tem-

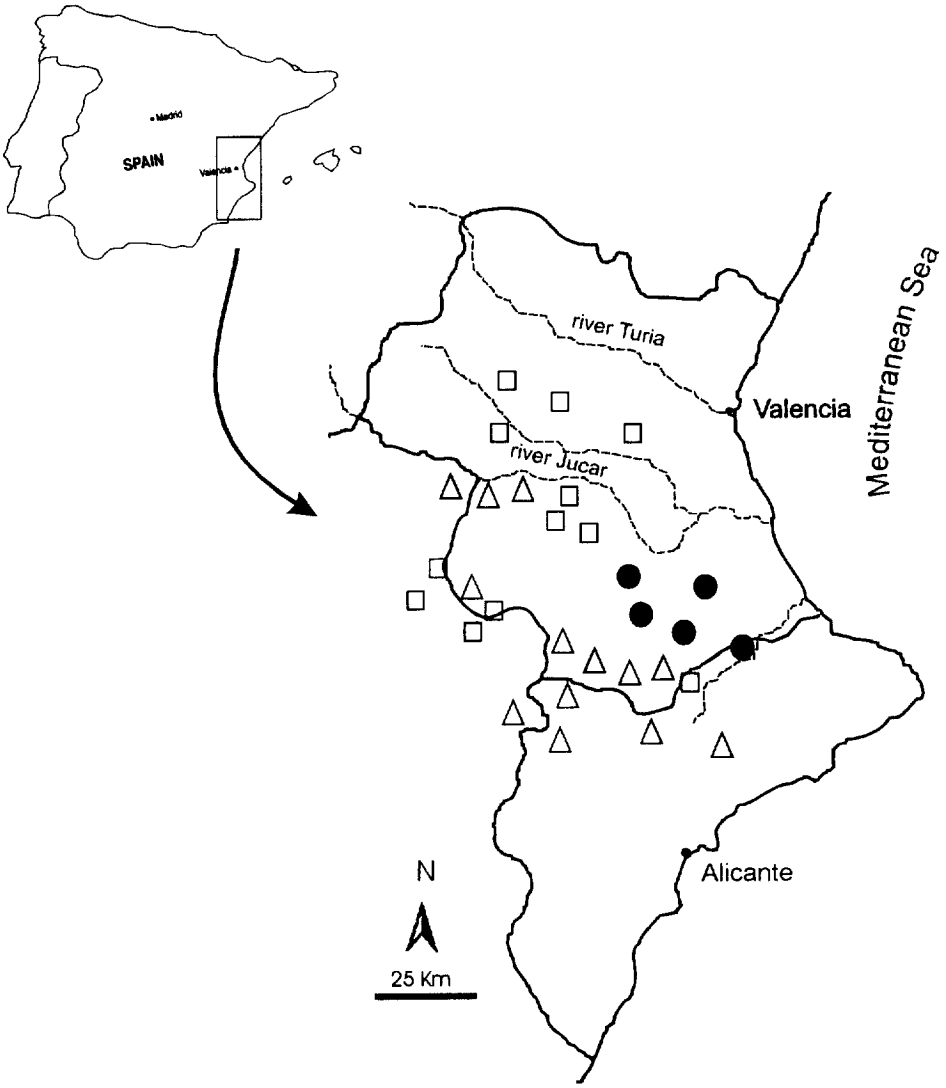

Fig. 4. Geographical distribution of populations studied and relative position of the chemotypes: □, Chemotype *p*-cymene carvacrol γ-terpinene; ●, chemotype *p*-cymene thymol; △, chemotype *p*-cymene carvacrol.

peratures were: injector 250°, detector 200°. Energy of the electronic gas has been of 70 eV and the quantification of the different components of the essential oil was accomplished with a GC endowed with the same capillary column and temperatures program that the GC-MS. Compounds were identified by the comparison of their retention times with those of authentic samples and GC-MS.

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