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ESSENTIAL OIL VARIABILITY OF *THYMUS ZYGIS* GROWING WILD IN SOUTHEASTERN SPAIN

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Key Word Index—*Thymus zygis* ssp. *sylvestris*; *T. zygis* ssp. *gracilis*; *T.* × *enicensis*; *T.* × *monrealensis*; nothosubsp. *garcía-vallejoi*; Lamiaceae; essential oil; thymol; linalool.

Abstract—Variations in the essential oils of *Thymus zygis* ssp. *sylvestris* and *T. zygis* ssp. *gracilis* growing in southeastern Spain were examined. Some hybrids with other species of thyme were detected, and the chemical influence of both parentals was demonstrated. The most common chemotype was thymol, although a pure linalool chemotype was recorded.

INTRODUCTION

Thymus zygis L. is a widespread endemic plant in the Iberian Peninsula with three subspecies recognized by Morales [1]. Two of them can be found in the Spanish southeast (Fig. 1), namely ssp. *sylvestris* (Hoffmanns & Link) Brot. ex Coutinho ($2n = 56$) and ssp. *gracilis* (Boiss.) R. Morales ($2n = 28$). Apart from their distribution area and different number of chromosomes, the subspecies differ mainly in the denser indumentum of the ssp. *sylvestris* and the more erect stems of ssp. *gracilis*. The latter is commonly collected from the field for traditional home use. In addition, its leaves are exported and, to a lesser extent, its essential oil is extracted.

Southeastern Spain has not been well represented in previous reports on the essential oils of *T. zygis*. Fernandes Costa [2], Ribeiro and co-workers [3-5] and Rodrigues and Ribeiro [6] demonstrated the presence of eight chemotypes (linalool, thymol, carvacrol, geraniol/geranyl acetate, 1,8-cineole/linalool, 1,8-cineole/thymol, linalool/thymol and 1,8-cineole/linalool/thymol) in Portuguese *T. zygis* ssp. *sylvestris*. Morales [1] examined three samples of *T. zygis* ssp. *sylvestris* from the centre of Spain, and found a thymol chemotype and two mixed thymol/borneol chemotypes. The same subspecies when studied by Mateo *et al.* [7] showed a predominant thymol chemotype with some samples containing nearly 15% of 1,8-cineole or camphor.

To date, studies on *T. zygis* ssp *gracilis* [1, 7-9] have always revealed one phenolic chemotype (mostly thymol, sometimes carvacrol) with smaller quantities of borneol and camphor (7-10%).

Several authors have shown that the essential oil composition of several aromatic species depends on factors which condition the general status of the plant. Vokou and Margaris [10] found a seasonal variation in oil concentration for *T. capitatus*, *Satureja thymbra* and *Teucrium polium* which reflects an important adaptive strategy towards summer drought. This explanation is not applicable to *Rosmarinus officinalis* under natural conditions [10, 11], this species having a higher essential oil content when grown with the addition of fertilizers [12].

For *Salvia officinalis*, Grella and Picci [13] obtained an annual variation in essential oil similar to the previously described species, and Maffei and Scannerini [14] state that the oil composition of *Mentha* × *piperita* is affected by the availability of photosynthetic NADPH, that is, by the stage of development. When micropropagated, this species has a higher menthol content, but a lower oil percentage, than when it is vegetatively propagated in the first year under field cultivation conditions [15].

As regards *T. zygis*, no specific work has been carried out on the relationship between season and the plant's essential oil content. In the present study, using samples taken at full bloom, a certain variability in the proportion of precursors/final compounds was found, reflecting the environmental conditions when each sample was collected.

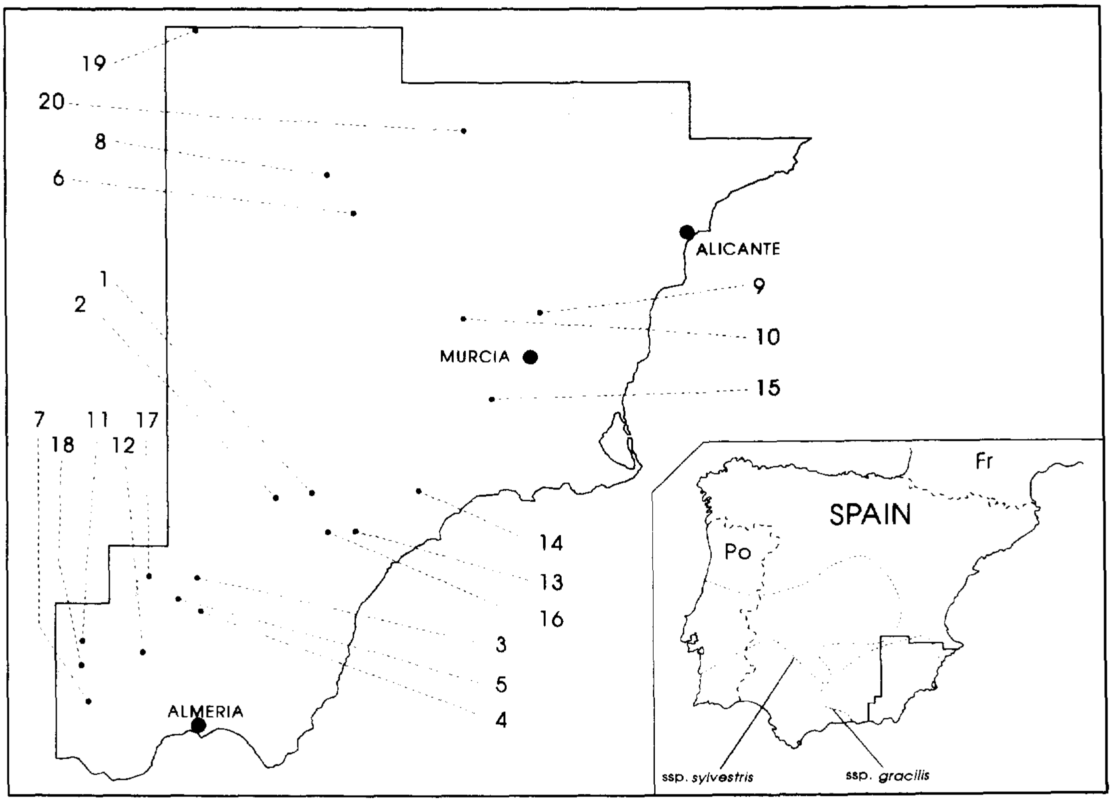

Fig. 1. Localization of populations in the study area. Approximate dispersal area of *T. zygis* ssp. *gracilis* and *T. zygis* ssp. *sylvestris*.

The aim of the present study was to investigate if the species' essential oil content is affected by bioclimate and ecology. For that purpose, 20 different sampling sites comprising 46 individuals were chosen (Table 1). Altitude ranged from 200 to 1400 m, covering the thermomediterranean, mesomediterranean and supramediterranean [16] bioclimatic belts, the semi-arid and dry [16] ombroclimates and a broad range of lithological conditions from calcareous to siliceous, and sometimes influenced by limestone. In addition, *T. zygis* easily hybridizes with some other species of thyme when the flowering periods overlap. In some of the selected localities there were one or two species growing side by side with *T. zygis*, and in a few of them the interaction was chemically noticeable.

Table 1. Field plots and number of samples taken

	Sampling sites	Samples
1	Puerto Santa María	1
2	Salientes	1
3	Bayarque	2
4	Velefique	2
5	Las Morcillas	2
6	Cenajo	2
7	Cortijo Chapina	3
8	Liétor	2
9	Santomera	2
10	Villanueva Río Segura	2
11	Fiñana	2
12	Ocaña	2
13	Enmedio	2
14	Campico López	2
15	Carrascoy	3
16	Urcal	2
17	Alcóntar	2
18	Láujar de Andarax	3
19*	Jardín	6
20*	Fuente Alamo	3

* Samples taken for *T. zygis* ssp. *sylvestris*.

RESULTS AND DISCUSSION

The variability in essential oils is represented at Fig. 2 by means of a cluster analysis. There are three major groups of individuals: (a) a group with a thymol chemotype and a variable quantity of the precursors *p*-cymene and γ-terpinene, (b) a group with a mixture of phenolic and non-phenolic compounds, and (c) a group with linalool as the major component. Table 2 shows the GC results for the most representative individuals within these groups.

Group (a) includes individuals with a thymol chemotype, the main variation being the greater or lesser presence of precursors (*p*-cymene, γ-terpinene). Carrascoy 3 was taxonomically identified as *T.* × *enicensis* Blanca (= *T. zygis* ssp. *gracilis* × *T. hyemalis*). Since both its parentals are a thymol chemotype, this hybrid is

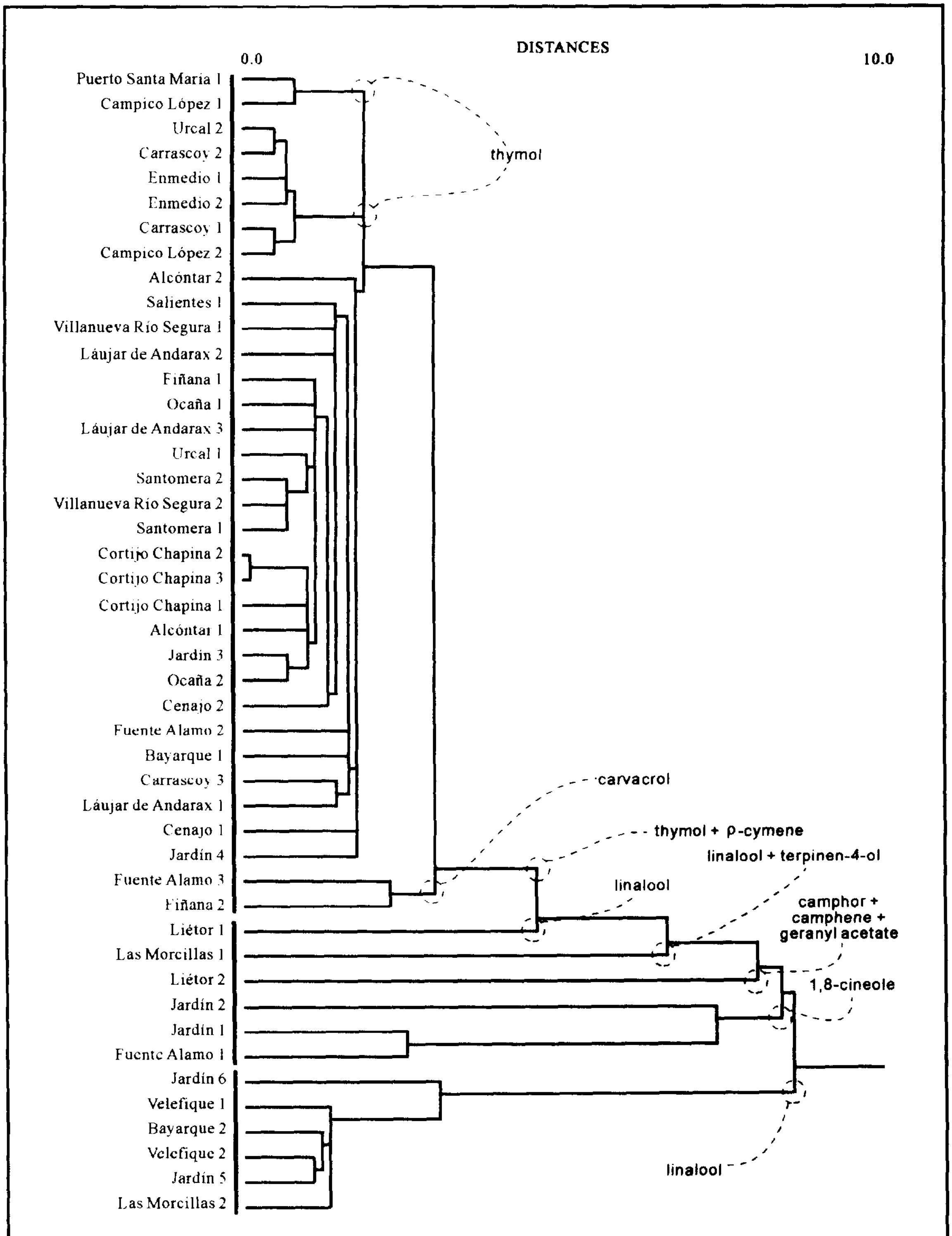

Fig. 2. Cluster analysis of the 46 individuals studied with remarks on the essential oil component(s) that characterize the major subgroups.

chemotaxonomically indistinguishable from them, and to date, its identification is exclusively based upon morphological features. Fiñana 1 displays a mixed thymol/carvacrol chemotype (25.45/22.79%), as occurs with Fuente Alamo 3 (24.3/18.20%) for the subspecies *sylvestris*.

Group (b) is chemically diverse due to its taxonomical diversity; (b1) samples from Liétor may reflect interac-

Table 2. Principal chemotypes found for *T. zygis* ssp. *gracilis* and *T. zygis* ssp. *sylvestris*

	ssp. *gracilis*						ssp. *sylvestris*			
Taxonomy*	1	2	3	4	5	6	7	8	9	10
Plot number	15	1	5	5	8	11	19	19	19	20
Individual number	3	1	1	2	2	1	2	3	6	3
α-Thujene	—	—	0.01	—	0.73	0.04	3.23	0.08	0.44	1.27
α-Pinene	3.08	0.04	4.77	0.10	7.76	0.89	0.24	2.28	—	0.73
Camphene	0.45	0.03	0.49	0.12	18.79	1.44	3.58	1.76	0.06	0.16
β-Pinene	0.11	0.04	0.34	0.04	1.14	0.18	2.25	0.29	0.66	0.07
Sabinene	0.36	—	0.05	—	0.13	0.05	0.02	0.06	0.19	1.22
Myrcene	0.72	0.08	3.14	0.08	7.98	0.85	1.72	0.09	0.20	1.67
α-Terpinene	1.38	0.02	0.07	—	0.08	0.86	0.24	1.91	0.03	0.34
Limonene	1.63	0.09	3.40	0.04	3.03	0.37	18.97	0.13	—	0.21
1,8-Cineole	0.27	1.02	0.08	0.11	0.22	0.26	34.50	1.54	16.13	0.02
β-*cis*-Ocimene		—	—	—	0.24	—	—	1.14	0.28	—
γ-Terpinene	9.29	0.36	0.15	0.26	1.45	6.34	1.52	10.95	0.18	10.89
p-Cymene	36.41	2.95	13.44	0.32	1.06	18.80	6.69	27.63	0.24	28.15
Terpinolene	0.08		0.04	0.04	0.11	0.08	0.79	0.15	—	0.10
trans-Sabinene hydrate	0.15	0.36	1.34	0.04	—	0.26	0.37	0.22	1.71	0.56
Camphor	0.79	0.56	0.11	0.07	14.52	0.27	0.19	0.10	1.35	0.82
Linalool	2.48	7.89	28.64	91.40	4.96	1.13	6.75	5.10	72.95	3.56
Linalyl acetate	—	0.06	1.63	0.68	0.11	0.16	0.07	0.04	0.52	0.04
Isobornyl acetate	0.68	1.17	0.40	0.08	1.37	0.68	0.99	1.04	0.36	0.34
Terpinen-4-ol	0.13	1.60	17.02	0.22	1.70	0.05	0.24	1.15	0.03	2.27
β-Caryophyllene	0.13	0.12	0.18	0.54	1.96	—	1.86	0.36	0.28	0.03
δ-Terpineol	—	0.11	0.40	0.03	—	0.85	0.33	0.03	0.40	0.05
Neral	1.25		—	—	—	—	—	—	—	—
α-Terpineol	1.26	0.08	0.47		—	0.05	1.07	0.04	0.02	0.21
Borneol	4.87	1.63	2.38	0.75		—	3.86	4.06	1.04	1.05
Verbenone	0.31	—		—	—	—	—	—	—	—
Geranial	0.13	—	—	—		—	—	—	—	—
Geranyl acetate	0.22	0.09	0.07	0.04	24.31	5.20	0.16	0.24	0.02	0.04
Citronellol	0.19	0.08	0.12	0.15	0.06	0.20	0.13	0.06	0.08	0.12
Geraniol	0.52	0.14	0.71	0.26	0.12	0.15	0.03	0.39	0.05	0.23
β-Caryophyllene ox.	0.15	0.07	0.77	0.17	0.10	0.51	0.55	0.63	0.20	1.29
Viridiflorol	0.43	0.08	0.80	0.04	0.86	—	0.06	—	0.11	0.05
Elemol	0.10	0.08	0.24	0.27	0.05	—	0.04	—	0.01	0.10
Spathulenol	0.15	0.28	0.40	0.07	—	0.14	0.08	—	0.11	0.16
Thymol	29.93	71.84	0.24	0.31	0.47	25.45	7.43	34.18	0.02	24.39
Carvacrol	1.47	3.34	0.12	0.05	0.35	22.79	0.03	1.66	0.03	18.20
α-Cadinol	—	0.05	0.12	0.02	0.18	0.01	0.06	0.04	0.02	0.03

* 1—*Thymus × enicensis* Blanca (= *T. zygis* ssp. *gracilis* × *T. hyemalis*); 2—ssp. *gracilis* chtyp. thymol; 3—ssp. *gracilis* chtyp. linalool/terpinen-4-ol; 4—ssp. *gracilis* chtyp. linalool; 5—*T. × monrealensis* Pau ex R. Morales nothosubsp. *garcia-vallejoi* Sánchez-Gómez *et al.* (= *T. zygis* ssp. *gracilis* × *T. vulgaris*); 6—ssp. *gracilis* chtyp. thymol/carvacrol; 7—ssp. *sylvestris* chtyp. 1,8-cineole/limonene; 8—ssp. *sylvestris* chtyp. thymol; 9—ssp. *sylvestris* chtyp. linalool/1,8-cineole; 10—ssp. *sylvestris* chtyp. thymol/carvacrol.

tions with *T. mastichina* (30.99% linalool in Liétor 1) and *T. vulgaris* (14.52% camphor and 18.79% camphene in Liétor 2), both species growing mixed with *T. zygis* ssp. *gracilis* at this locality. Liétor 1 shows no morphological similarity with *T. mastichina*, while Liétor 2 presented flower and leaf characteristics close to those of *T. vulgaris*. This suggests introgressive hybridization from *T. zygis* ssp. *gracilis* to *T. vulgaris*. (b2) Jardín 1 and Jardín 2 show high levels of 1,8-cineole that may be due to an interaction with *T. orospedanus* and *T. mastichina*, both of which are found at this sampling plot with *T. zygis* ssp. *sylvestris*. (b3) Las Morcillas 1 is a variation of the linalool group, with a 17.02% terpinen-4-ol.

Group (c) has a linalool chemotype. Velefique and Las Morcillas sampling plots are situated near the top of the Los Filabres mountain range. The *T. zygis* found growing there is at present being studied and shows several differences with the surrounding ssp. *gracilis*: it is a tetraploid taxon ($2n = 56$; this needs confirmation) with later blooming and fructification, shorter stems, denser filotaxis, more whitish corolla, darker green leaves and pale yellow essential oil glands (with linalool), which lack the reddish colour that characterizes them when their content is mainly phenols. At Filabres, a pure linalool chemotype not previously reported for *T. zygis* has been found. Samples from Bayarque are also within the Filabres range, although at a lower altitude, and here the transition of ssp. *gracilis s. str.* to the linalool chemotype is of interest. The position of Jardín 6 in Fig. 2 reflects the influence mentioned for group (b) with a stronger linalool content.

Figure 3 shows the results for the principal component analysis (PCA). Factor 1 separates phenolic from non-phenolic samples, and factor 2 distinguishes the linalool chemotype. The phenolic group stands in the upper-right part with minimal differences among most individuals. The position of Jardín 3 (thymol chemotype of ssp. *sylvestris*) within the group is shown. The linalool chemotype group shows the most negative values of factor 2. Liétor 1 stands alone due to its thymol and linalool content. Liétor 2 and Fuente Alamo 1 are characterized by their non-phenolic chemotype. The percentage of 1,8-cineole separates Jardín 1 and Jardín 2 from the rest.

Figure 4 illustrates relationships among compounds identified in the essential oils. Only those reaching at least 5% in one sample are presented. The strong negative correlation between linalool and the phenols and their precursors is clear. Not so marked, but clearly visible on dimension 2, is the disjunction between initial (*p*-cymene and γ-terpinene) and final (thymol and carvacrol) compounds of the phenolic biosynthetic pathway (the best represented in the samples analysed). 1,8-Cineole, borneol, geranyl acetate, etc., are individually isolated and far from the two main chemotypes showing alternative pathways that may be present due to interactions with other species of thyme (*T. vulgaris*, *T. mastichina*, *T. orospedanus*) growing close to the samples taken.

There is no correlation between essential oil components and bioclimatic variables (altitude, bioclimatic belt, ombroclimate) at a 99% confidence interval. If this percentage is fixed at 95, two positive and five negative correlations are found (Table 3). Phenols are mainly produced at lower altitudes, in warmer and less humid biotopes.

In conclusion, a thymol chemotype is predominant in the study area, whereas a pure linalool chemotype is found in a very limited area. Mixed chemotypes are restricted to some places where two different species of thyme coexist. All of this deals with ssp. *gracilis*. Ssp. *sylvestris* has a thymol chemotype. The contents of 1,8-cineole and linalool are due to interactions with *T. vulgaris* and *T. mastichina*, respectively, which are further supported by intermediate morphological features.

EXPERIMENTAL

Plant material. Aerial parts of flowering *T. zygis* ssp. *sylvestris* (2 localities) and *T. zygis* ssp. *gracilis* (18 localities) were collected from sites in the Spanish southeast (Fig. 1, Table 1) from May to July of 1990 to 1993. The number of individuals taken (1–6) from each locality depended on the morphological variability observed. The plant material was dried at room temp. and steam distilled for 2 h in a Clevenger-type apparatus. Essential oils were kept in sealed glass tubes at 4° until analysis. Voucher specimens from each locality are kept at the Herbarium of the Murcia University (MUB).

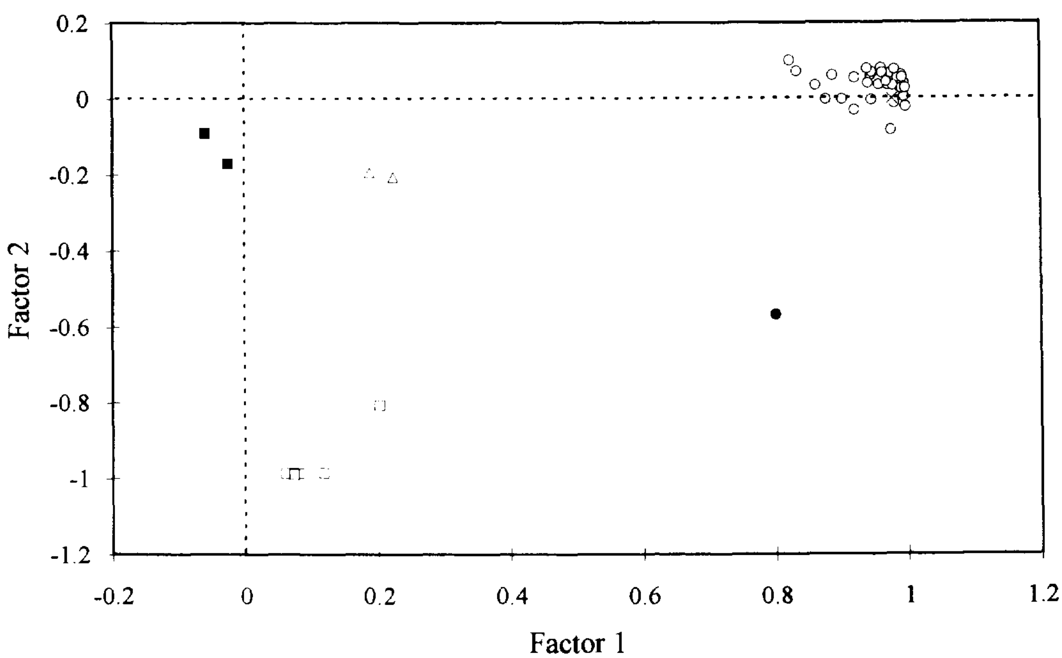

Fig. 3. Principal component analysis. □, linalool group; ●, Liétor 1; △, Jardín 1 and Jardín 2; ■, Liétor 2 and Fuente Alamo 1; ○, phenolic group (the position of a ssp. *sylvestris* sample, Jardín 3 (×), within it is marked).

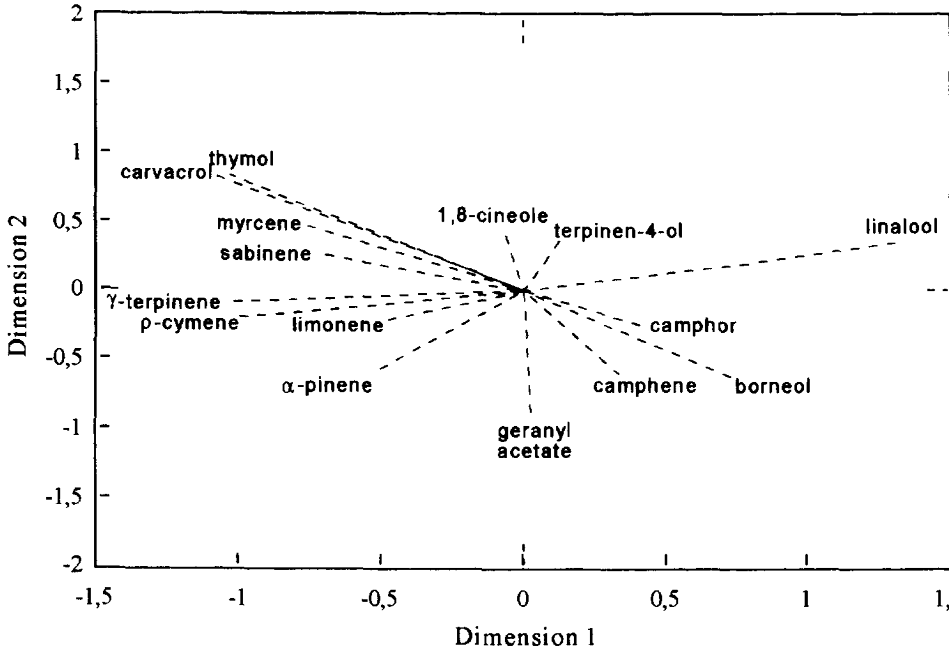

Fig. 4. Multidimensional scaling analysis. Only compounds with 5% in at least one sample are represented.

Table 3. Statistically significant correlations between essential oil components and bioclimatic variables (altitude, bioclimatic belt, ombroclimate). All correlations have a confidence level of 95%. No correlation was found at 99%

Pair of variables	r
Altitude/δ-terpineol	0.40
Altitude/thymol	− 0.46
Altitude/carvacrol	− 0.45
Bioclimatic belt/thymol	− 0.40
Ombroclimate/δ-terpineol	0.36
Ombroclimate/thymol	− 0.45
Ombroclimate/carvacrol	− 0.41

GC analysis. GC was carried out on a Hewlett Packard Series II 5890 gas chromatograph equipped with a FID using a 25 m × 0.2 mm 'Carbowax 20M' capillary column with the following temp. program: 70° isothermal for 1 min, then an increasing rate of $10° \text{ min}^{-1}$ up to 90°, isothermal for 1 min, and again an increasing rate of $10° \text{ min}^{-1}$ up to 210°. Injector and detector temp. was 250°. Carrier gas was He at 1 ml min^{-1}. Peak areas and concns were calculated with a Hewlett Packard 3396A integrator. Individual components were identified by IR, R_t comparison with pure standards, and GC-MS. Percentage of identified compounds at each sample ranged from 90 to 99%.

Statistical analysis. All data were statistically processed with Systat 5 PC software. Trace quantities ($< 0.01\%$) were considered as zero value. Analysis included: (a) cluster analysis to establish the different groups/chemotypes within the individual essential oils. (b) PCA to check for partition among the identified compounds. (c) Spearman correlation between essential oil components and bioclimatic variables (altitude, bioclimatic belt, ombroclimate) to state relations with the environment. The bioclimatic belt and ombroclimate cited for each locality is based on the work by Rivas-Martinez [16]. (d) Multidimensional scaling was based on a Spearman correlation matrix to see the relationship between the different compounds.

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