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Essential oil composition is related to the natural habitats: *Coridothymus capitatus* and *Satureja thymbra* in NATURA 2000 sites of Crete

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Abstract

The study of essential oils obtained from *Coridothymus capitatus* and *Satureja thymbra* collected from different natural habitat types of 11 NATURA 2000 sites scattered all over Crete has shown that they are characterized either by a high amount of carvacrol (up to 75.7%) or thymol (up to 65.6%) or by a more or less equal amount of the two phenols. The results of a discriminant analysis with predefined groups the natural habitat types wherefrom the plants were collected have shown that the oils of both species collected from the dry dwarf-shrub formations of the lowland have a high carvacrol content whereas those collected from the more mesic timber or highland formations have a high thymol content. Furthermore, the results of this study introduce the use of natural habitat unit as a tool for the assessment of essential oil variation.

Keywords: *Coridothymus capitatus* Reichenb. fil.; *Satureja thymbra* L.; Labiatae; Crete; NATURA 2000; Natural habitat type; Essential oils; Carvacrol; Thymol

1. Introduction

NATURA 2000 is a coherent European ecological network of special areas of conservation set up for the implementation of Directive 92/43/EEC. The aim of the Directive was to contribute towards ensuring bio-diversity through the conservation of natural habitats and of wild fauna and flora in the European territory of the Member States. The NATURA 2000 network is composed of sites hosting the natural habitat types and habitats of the species listed in Annexes I and II of the Directive. A NATURA 2000 site is a geographically defined area whose extent is clearly delineated. The natural habitats of each site are distinguished by geographic, abiotic and biotic features. The habitat mapping within all the NATURA 2000 sites follows a common codification and description of each habitat type, the latter largely based on the dominant vegetation of an area (Commission of Europe Communities (CEC), 1991; Council of Europe, 1992). Thus, a functional new index for the estimation of the environmental complexity is provided, for the areas included in the network.

On the basis of the criteria set out in the Directive 92/43/EEC and relevant scientific information, 296 sites including natural habitat types of European interest have been selected in Greece. Among them 34 sites are located on the island of Crete (Dafis et al., 1996; Hellenic Ministry for the Environment, Physical Planning & Public Works, 2005).

Coridothymus capitatus (L.) Reichenb. fil. [syn. *Thymus capitatus* (L.) Hoffmanns. & Link] and *Satureja thymbra* L. are Mediterranean aromatic shrubs, abundantly found all over Crete (Karousou, 1995). Both plants produce

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essential oils rich in phenolic compounds and are used as oregano spices (Kokkini et al., 2003), implying that carvacrol is the dominant phenol in their essential oils. Although this holds true for the majority of *C. capitatus* oils studied, scattered publications report thymol or both phenols as main oil constituents (Ravid and Putievsky, 1983; Cosentino et al., 1999; Gören et al., 2003). On the other hand carvacrol – rich *S. thymbra* oils are not frequently reported (Kokkini and Vokou, 1989; Lagouri et al., 1993; Sokovic et al., 2002; Gören et al., 2004; Fleisher and Fleisher, 2005).

A number of studies in the phenol-rich Labiatae species *Thymus vulgaris* L. (Gouyon et al., 1986), *T. piperella* L. (Boira and Blanquer, 1998) and *Origanum vulgare* L. (Vokou et al., 1993) have shown that the preponderance of carvacrol or thymol in their essential oils is associated to climatic conditions.

In an attempt to find out if any relation exists between *C. capitatus* and *S. thymbra* essential oil composition and the natural habitat type wherefrom the plants were collected, the present paper studies the oils of wild growing plants in 11 NATURA 2000 sites scattered all over Crete.

2. Results and discussion

The 26 oils analyzed were characterized by the four compounds involved in the phenolic biosynthetic pathway, viz., γ-terpinene, *p*-cymene, thymol and carvacrol. The sum of the four compounds constitutes the bulk of the essential oil ranging from 78.8% to 87.1% for *C. capitatus* and from 75.9% to 84.9% for *S. thymbra*. However, a considerable quantitative variation has been observed (Table 1, Figs. 1A and B). In both taxa the highest fluctuations have been found in the amounts of thymol (0.2–62.8% of the total oil for *C. capitatus* and 0.1–65.6% for *S. thymbra*) and carvacrol (8.3–75.7% for *C. capitatus* and 5.2–66.5% for *S. thymbra*).

Our previous findings on other Labiatae widespread in Crete, have shown that the variation of their quantitative oil composition follows the geographic direction from the western to the eastern part of the island (Kokkini et al., 1997a; Karousou et al., 1998).

The results of the PCA analysis applied to examine if any similar geographic pattern exists, revealed that the oils studied form three groups not related to the range of the two species. Essential oils obtained from plants growing in West Crete are grouped with those from plants growing to the central and/or eastern part of the island (Figs. 1 and 2). The variables mainly responsible for the ordination are thymol and carvacrol (ordination scores 0.91 and −0.99, respectively along component 1 and −0.41 and 0.02 along component 2), which follow an almost opposite trend (Fig. 2). Component 1 accounts for the 49.65% of the total variance and component 2 accounts for a further 30.61%.

The Cretan climate being in general typical Mediterranean, is locally differentiated by altitude and exposure (Pennas, 1977; Hager, 1985). As a result several natural habitat types are included within a single NATURA 2000 site (Table 2).

In order to find out if the essential oil variation of *C. capitatus* and *S. thymbra* is related to the different habitats, a discriminant analysis using as pre-defined groups the natural habitat types where the plants were collected was applied. The results are presented in Fig. 3, by the ranges of their discriminant scores along function 1 (accounted for the 94.7% of the total variance), which mainly consists of the percentages of carvacrol and thymol (standardized coefficients 1.01 and −0.29, respectively). The total percentage of the correctly a priori classified cases is 80.8%. The three distinct groups shown in Fig. 3 are the same with those provided by the PCA analysis and can be described as follows:

Group A: Essential oils obtained from plants grown in *Sarcopoterium spinosum* phryganas. It consists of *C. capitatus* and *S. thymbra* oils (samples 1, 2, 5, 8–13,

Table 1
Qualitative and quantitative composition of *Coridothymus capitatus* (1–13) and *Satureja thymbra* (14–26) plants collected from different localities of Crete

	Locality												
	1	2	3	4	5	6	7	8	9	10	11	12	13
γ-Terpinene	2.9	5.3	6.1	8.7	12.1	5.0	3.7	12.5	1.2	5.0	6.9	6.7	7.6
p-Cymene	7.8	8.1	9.2	7.5	9.2	6.1	10.1	12.9	8.5	7.0	7.0	11.7	8.8
Thymol	0.2	1.8	62.8	25.9	0.5	62.6	45.5	0.5	1.7	0.5	0.6	0.7	0.6
Carvacrol	72.3	70.6	8.9	36.7	62.4	8.3	23.5	55.8	75.7	74.2	69.7	65.1	69.4
Total concentration in essential oil (%)[a]	83.2	85.8	87.0	78.8	84.2	82.0	82.8	81.7	87.1	86.2	84.2	84.2	86.4
	14	15	16	17	18	19	20	21	22	23	24	25	26
γ-Terpinene	16.2	22.6	4.4	17.0	14.3	13.9	20.0	16.1	9.7	11.6	16.6	5.7	19.9
Cymene	14.8	8.7	7.2	5.9	7.7	7.0	11.2	12.8	15.0	7.9	6.1	5.5	8.5
Thymol	38.9	18.6	65.6	19.9	17.8	21.1	0.8	42.1	13.0	0.3	0.6	0.4	0.1
Carvacrol	6.0	27.6	7.7	40.0	37.5	37.5	48.2	5.2	38.4	61.8	54.4	66.5	53.6
Total concentration in essential oil (%)[a]	75.9	77.5	84.9	82.8	77.3	79.5	80.2	76.2	76.1	81.6	77.7	78.1	82.1

For the localities see Fig. 1 and Table 2.
[a] The remainder consists of minor constituents which do not present any significant quantitative differences among the localities.

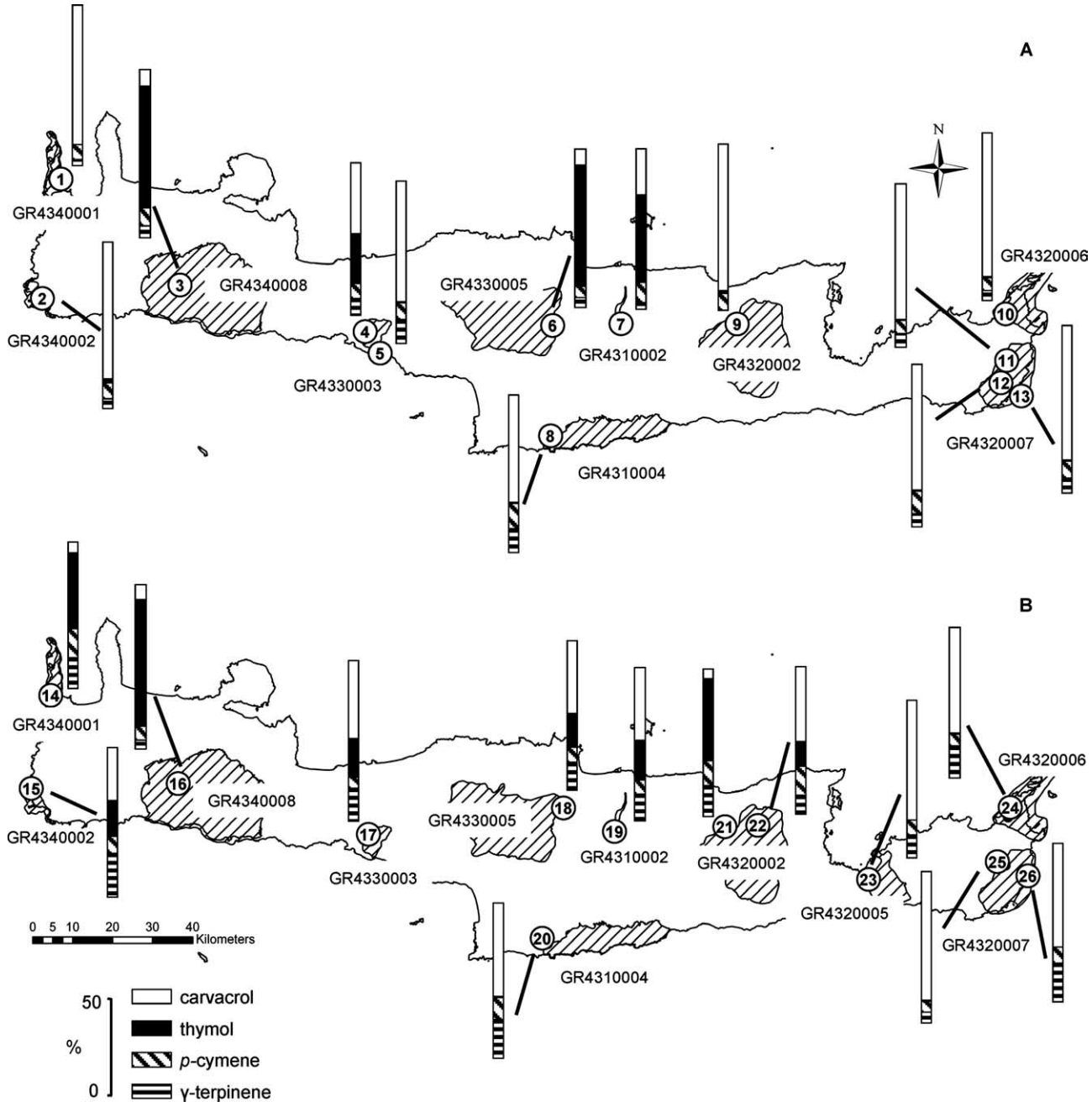

Fig. 1. Percentages of γ-terpinene, *p*-cymene, thymol and carvacrol in the total essential oil of *Coridothymus capitatus* (A) and *Satureja thymbra* (B) plants; the numbers correspond to Cretan localities wherefrom the analyzed samples were collected (see also Table 2).

20, 23–26) having carvacrol as main component (48.2–75.7%), while thymol was traced in negligible amounts (0.1–1.8%) (Figs. 1 and 2).

Group B: Oils from plants grown in endemic phryganas of the *Euphorbio–Verbascion*, Arborescent matoral with *Juniperus* spp. and Eastern garrigues. It comprises *C. capitatus* and *S. thymbra* oils (samples 4, 7, 15, 17–19, 22) having high amounts of both phenols (thymol 13.0–45.8%, carvacrol 23.5–40.0%) (Figs. 1 and 2), with a thymol:carvacrol ratio 0.3–1.9.

Group C: Oils from plants grown in Greek kermes oak forests, *Olea* and *Ceratonia* forests and endemic oro-Mediterranean heaths with gorse. It comprises oils of both species (samples 3, 6, 14, 16, 21) with thymol prevailing over carvacrol (38.9–65.6% and 5.2–8.9%, respectively) (Figs. 1 and 2) at a ratio 6.5–8.5.

The above results suggest that the essential oil quantitative composition is related to the plants habitat. In particular they have shown that:

(i) Plants of a single species growing in the same natural habitat have similar oil composition. For example the essential oils of *S. thymbra* plants growing in

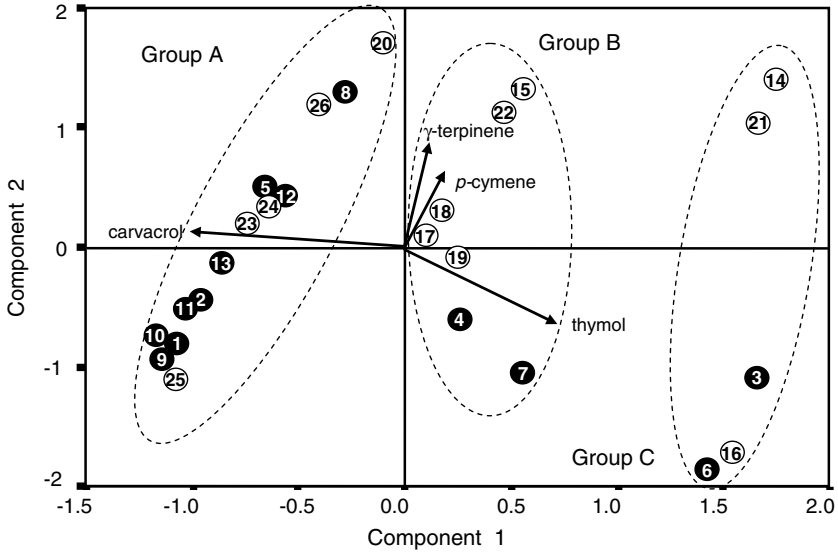

Fig. 2. Principal component analysis (PCA) ordination scores of *Coridothymus capitatus* (solid symbols) and *Satureja thymbra* (open symbols) oil samples. The numbers correspond to the localities of Table 2 and Fig. 1. Arrows indicate the ordination scores of the variables used.

Table 2
Localities wherefrom *Coridothymus capitatus* (1–13) and *Satureja thymbra* (14–26) were collected

Locality	Coordinates	NATURA 2000 Site	Habitat type
1	35° 31′ 43″ N/23° 35′ 40″ E	GR4340001	5420
2	35° 16′ 13″ N/23° 32′ 27″ E	GR4340002	5420
3	35° 18′ 30″ N/23° 55′ 88″ E	GR4340008	4090
4	35° 11′ 23″ N/24° 27′ 42″ E	GR4330003	5430
5	35° 09′ 01″ N/24° 28′ 09″ E	GR4330003	5420
6	35° 12′ 46″ N/24° 57′ 12″ E	GR4330005	934A
7	35° 12′ 37″ N/25° 08′ 27″ E	GR4310002	5340
8	34° 57′ 18″ N/24° 56′ 48″ E	GR4310005	5420
9	35° 12′ 43″ N/25° 27′ 06″ E	GR4320002	5420
10	35° 15′ 54″ N/26° 12′ 44″ E	GR4320006	5420
11	35° 06′ 55″ N/26° 12′ 28″ E	GR4320007	5420
12	35° 03′ 09″ N/26° 12′ 42″ E	GR4320007	5420
13	35° 02′ 04″ N/26° 13′ 37″ E	GR4320007	5420
14	35° 30′ 36″ N/23° 34′ 05″ E	GR4340001	9320
15	35° 18′ 26″ N/23° 31′ 59″ E	GR4340002	5210
16	35° 18′ 20″ N/23° 55′ 07″ E	GR4340008	4090
17	35° 11′ 30″ N/24° 27′ 42″ E	GR4330003	5430
18	35° 15′ 26″ N/24° 58′ 57″ E	GR4330005	5340
19	35° 12′ 39″ N/25° 08′ 24″ E	GR4310002	5340
20	34° 57′ 13″ N/24° 56′ 48″ E	GR4310005	5420
21	35° 12′ 28″ N/25° 27′ 08″ E	GR4320002	934A
22	35° 12′ 46″ N/25° 31′ 08″ E	GR4320002	5340
23	35° 05′ 04″ N/25° 50′ 06″ E	GR4320005	5420
24	35° 13′ 20″ N/26° 12′ 60″ E	GR4320006	5420
25	35° 06′ 50″ N/26° 12′ 25″ E	GR4320007	5420
26	35° 05′ 46″ N/26° 15′ 27″ E	GR4320007	5420

For the localities see also Fig. 1. For the description of the habitat type see Section 3.

Sarcopoterium spinosum phryganas found in different NATURA 2000 sites are rich in carvacrol (samples 20 and 24, Figs. 1 and 2).

(ii) Plants of a single species growing in different habitat types have different oil composition. Thus, *C. capitatus* oils from plants growing in a single site (GR4330003) are rich either in both phenols (in endemic phryganas of the *Euphorbio–Verbascion*) or in carvacrol (in *Sarcopoterium spinosum* phryganas) (samples 4 and 5, Figs. 1 and 2).

(iii) Plants belonging to different species growing in the same habitat have oils with similar composition. Thus, *C. capitatus* and *S. thymbra* oils from plants growing in endemic oro-Mediterranean heaths with gorse (site GR4340008) are rich in thymol (samples 3 and 16, Figs. 1 and 2).

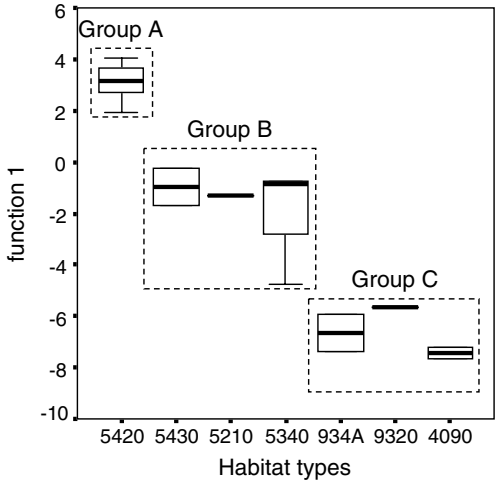

Fig. 3. Box plots of the discriminant scores of the oil groups provided by discriminant analysis (horizontal lines show the 25, 50 and 75th percentile, while outer limits of the whiskers min and max values). For the explanation of the habitat type codification see Section 3.

In concluding, the findings of the present paper have shown that a high carvacrol content is associated to the dry dwarf-shrub formations of the lowland (Group A) whereas a high thymol content to the more mesic timber

or highland formations (Group C) (Fig. 3). The relation between oil composition and the natural habitats wherefrom plants were collected suggests the use of natural habitat unit as a tool for the assessment and prediction of a single species essential oil variation.

3. Experimental

3.1. Plant material

C. capitatus and *S. thymbra* plants were collected during mid-summer from 26 localities scattered in 11 NATURA 2000 sites of the island of Crete (Table 2, Fig. 1A and B). *C. capitatus* plants were full blooming, while *S. thymbra* had developed fruits. Twenty individuals representing the local populations were collected from each locality. Voucher specimens are deposited in the Herbarium of the Laboratory of Systematic Botany and Phytogeography, Aristotle University of Thessaloniki (TAU).

The codification of the natural habitat types is according to the Council of Europe (1992), and Dafis et al. (1999) and their description according to the Commission of European Communities (1991):

4090 – Endemic oro-Mediterranean heaths with gorse: Primary cushion heaths of the high, dry mountains of the Mediterranean region, with low cushion – forming, often spiny shrubs (in Crete altitudinal range 1500–2500 m).

5210 – Arborescent matoral with *Juniperus* spp.: Mediterranean and sub-Mediterranean evergreen sclerophyllous bush and scrub organized around arborescent junipers.

5340 – Eastern garrigues: Shrubby formations, often low, of the meso- and occasionally supra-Mediterranean zones of Greece.

5420 – *Sarcopoterium spinosum* phryganas: Low, thorny formations of hemispherical shrubs of the coastal thermo-Mediterranean zone of Greece and its islands.

5430 – Endemic phryganas of the *Euphorbio–Verbascion*: Varied formations of supra- and oro-Mediterranean levels of Crete resulting from the broad contact between phryganas and hedgehog-heaths.

9320 – *Olea* and *Ceratonia* forests: Thermo-Mediterranean or thermo-Canarian woodland dominated by arborescent *Olea europaea* subsp. *sylvestris*, *Ceratonia siliqua*, *Pistacia lentiscus*, *Myrtus communis* or, in the Canary islands, by *Olea europaea* subsp. *cerasiformis* and *Pistacia atlantica*.

934A – Greek kermes oak forests: Arborescent *Quercus coccifera*-dominated formations of peninsular Greece, the Ionian and Aegean archipelagos and of Crete.

The identification of the habitat types wherefrom plants were collected is based on the maps provided by the Hellenic Ministry for the Environment, Physical Planning & Public Works (2005) and on personal observations.

3.2. Essential oil analysis

Aerial parts of the collected plants were air-dried at room temperature for 10 days. Then they were grossly pulverized and subjected to hydrodistillation for 2 h using a Clevenger apparatus. GC and GC/MS analyses were carried out according to a previously described procedure (Kokkini et al., 1997b).

3.3. Statistical analysis

Principal component analysis (PCA) and discriminant analysis were applied using the following variables: participation in the total oil of γ-terpinene, *p*-cymene, thymol and carvacrol.

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