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AUTUMN ESSENTIAL OILS OF GREEK OREGANO

STELLA KOKKINI,* REGINA KAROUSOU, ANTONIA DARDIOTI, NIKOS KRIGAS and TOM LANARAS†

Laboratory of Systematic Botany and Phytogeography and †Department of Botany, School of Biology, Aristotle University of Thessaloniki, 540 06 Thessaloniki, Greece

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Key Word Index—*Origanum vulgare* subsp. *hirtum*; Labiatae; Greek oregano; essential oils; seasonal variation; γ-terpinene; *p*-cymeme; carvacrol; thymol.

Abstract—The essential oils of *Origanum vulgare* subsp. *hirtum* plants collected in late autumn from six localities of three distinct geographic areas of Greece were analysed by means of GC and GC-mass spectrometry. A high quantitative variation was found in the amount of the four main components; γ-terpinene ranged from 0.6 to 3.6% of the total essential oil, *p*-cymeme from 17.3 to 51.3%, thymol from 0.2 to 42.8%, and carvacrol from 1.7% to 69.6%. Plants collected from the northern part of Greece were rich in thymol (30.3–42.8% of total oil), whereas those from the southern part of the country were rich in carvacrol (57.4–69.6% of total oil). Furthermore, comparison with the essential oils obtained from plants collected from the same localities in mid-summer shows noticeable differences in the total oil content and the concentration of the four main oil components. Copyright © 1997 Elsevier Science Ltd

INTRODUCTION

A number of studies have shown that *Origanum vulgare* subsp. *hirtum* (Link) Ietswaart (syn.: *O. hirtum* L., *O. heracleoticum* auct. non L.) is a very variable taxon both in morphological and in chemical terms. In particular, it has been found that the essential oil content of this taxon, commercially known with the vernacular name 'Greek oregano', ranges in Greece from 1.1 to 8.2% (ml 100 g^{-1} dry wt); the highest values have been recorded in the Aegean islands and in areas of mainland surrounding the Aegean Sea. Furthermore, it has been found that the essential oil composition of the wild-growing plants is characterized either by the dominant presence of carvacrol or thymol or by almost equal amount of these phenols. In all cases, the two monoterpene hydrocarbons, γ-terpinene and *p*-cymeme, were constantly present in all essential oils analysed, but always in lower amounts than those of the two phenols [1–4].

Published information thus suggests that the pattern of geographic variation of the wild-growing Greek oregano plants follows the climatic changes in the country. It should be noted that the above conclusion was solely based on results obtained from plants collected during summer, when the plants were fully flowering. Consequently, there are no data for the chemical profile of these plants in other seasons.

The present paper is part of a project aiming to introduce the most promising *O. vulgare* subsp. *hirtum* plants, i.e. plants with a high essential oil content and high amount of carvacrol or thymol, in experimental cultivation fields. We collected wild plants during late autumn—an appropriate season for rhizome propagation—from six selected localities of three distinct geographic areas of Greece. Their essential oil content, qualitative and quantitative composition are presented and compared with those known from plants, harvested in the summer.

RESULTS AND DISCUSSION

The essential oil yield of *O. vulgare* subsp. *hirtum* plants collected during late autumn from the three geographic areas of Greece (Fig. 1) ranged from 1.0 to 3.1% (ml 100 g^{-1} dry wt) (Table 1). It should be noted that the summer values of oil yield obtained from plants collected from the same areas were much higher (4.8–8.2%) [1–4]. A significant decrease of essential oil content in late autumn has also been reported in non-flowering *O. vulgare* subsp. *hirtum* plants cultivated in Israel [5].

The main components of the six essential oils analysed were γ-terpinene, *p*-cymeme, thymol and carvacrol. The essential oils obtained from plants grown in South Peloponnese and on the island of Crete are characterized by the presence of higher levels of carvacrol (more than 57% of total essential oil), whereas those obtained from the Athos peninsula are lower in

* Author to whom correspondence should be addressed.

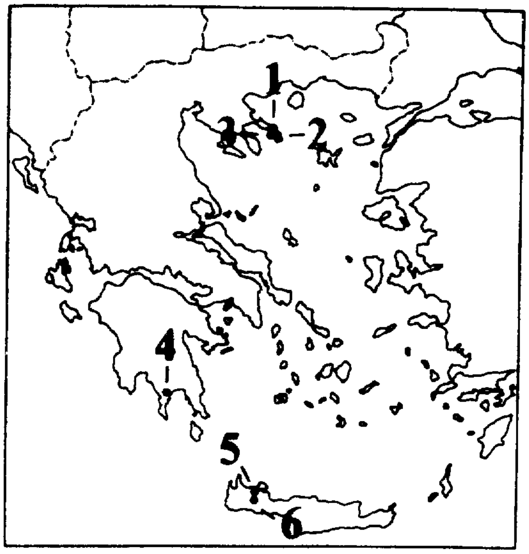

Fig. 1. Map of Greece showing the locations where *Origanum vulgare* subsp. *hirtum* plants were collected. Athos peninsula: (1) NE of the town of Karyes, behind the Skiti of Agios Andreas (40° 15′ 30″ N/24° 14′ 50″ E), at an altitude of 350 m, 17 November 1995; (2) S of the town of Karyes, 1 km SE of the Monastery of Koutloumousiou (40° 15′ N/24° 16′ 20″ E), 270 m, 17 November 1995; (3) Town of Karyes (40° 15′ 20″ N/24° 14′ 20″ E), 500 m, 18 November 1995. Peloponnese: (4) Village of Marathea, 26 km SW of the city of Gythion (36° 44′ N/22° 29′ 15″ E), s.l., 20 November 1995. Island of Crete: (5) S of the village of Perivolia (35° 29′ N/23° 59′ 30″ E), 130 m, 20 November 1995; (6) Village of Meskla (35° 24′ 20″ N/23° 57′ 30″ E), 170 m, 20 November 1995.

carvacrol (<2.5% of total oil). In the latter case, thymol is the predominant phenol, ranging from 30.3 to 42.8% of the total oil. These results support our previous findings which have shown that *O. vulgare* subsp. *hirtum* plants grown in South Pelopennese and on the island of Crete produce an 'oregano'-type essential oil, whereas those from Athos peninsula have a 'thyme'-type oil [1–3, 6]. Along with the two phenols, the amount of γ-terpinene and *p*-cymeme ranges from 0.6 to 3.6% and from 17.3 to 51.3% of the total oil, respectively (Table 1).

Taking into account the summer and autumn values of *O. vulgare* subsp. *hirtum* essential oil yield and the amount of the four major oil constituents, the plants from the three geographic areas can be grouped as shown in Fig. 2. Plants collected in autumn are mainly distinct from those of the summer by their lower essential oil yields, lower amount of γ-terpinene and much higher amount of *p*-cymeme. The widest range of variation between summer and autumn essential oil features has been recorded in the plants grown in Athos peninsula. This area lies very close to the transitional Continental Mediterranean climatic zone of Greece, which is characterized by sharper differences between the two seasons in a number of climatic parameters, such as the mean monthly temperature, the amount of precipitation and the duration of sunshine, than South Peloponnese and Crete. The last two areas are located within the Real Mediterranean climatic zone, which is characterized by milder fluctuations of the above parameters during the year [4, 7].

The differences found in the amount of each one of the four main components of *O. vulgare* subsp. *hirtum* essential oils between the summer and autumn plants are shown in Fig. 3. The most marked difference is the increased amount of *p*-cymeme in autumn; its concentration ranges from 17.3–26.9% of the total oil in plants from South Peloponnese and Crete (instead of 4.0–9.5% found in the summer plants) to 37.1–51.3% of the oil in plants from Athos peninsula (instead of 12.0–12.2% in the summer). An increase in the amount of *p*-cymeme during the growth of the plant has been found by Maarse [8], who studied the changes in essential oil composition of *O. vulgare* subsp. *vulgare*, a phenol-poor taxon.

Despite the striking quantitative differences of the major oil components, their sum (γ-terpinene + *p*-cymeme + thymol + caravacrol) is almost equal in the essential oils of different geographic origin, as well as in the different seasons, ranging from 85.0 to 96.8%. This suggests that the four compounds are biosynthetically very close and supports the findings for the formation of thymol from γ-terpinene via *p*-cymeme in *Thymus vulgaris* essential oil [9]. It should be noted that the two monoterpene hydrocarbons are very common constituents of all oregano- or thyme-type essential oils [6, 10, 11]. However, high concentrations of *p*-cymeme similar to those found in *O. vulgare* subsp.

Table 1. Essential oil yield, qualitative and quantitative composition from Greek oregano plants collected in autumn (numbers of sampling sites correspond to the locations shown in Fig. 1)

	Collection sites					
Compound	1	2	3	4	5	6
γ-Terpinene	3.6	0.6	2.2	1.8	2.7	2.8
p-Cymene	37.1	51.3	38.7	17.3	26.9	17.7
Thymol	42.8	30.3	41.4	4.1	0.3	0.2
Carvacrol	2.4	2.5	1.7	67.7	57.4	69.6
Total concentration in essential oil (%)*	85.9	84.7	84.0	90.9	87.3	90.3
Essential oil yield (ml 100 g^{-1} dry wt)	1.3	1.0	1.3	3.1	1.0	1.6

* The remainder consists of 32, 33, 32, 33, 34 and 34 components in each site. All of them are minor constituents and do not present any statistically significant quantitative difference between the sites.

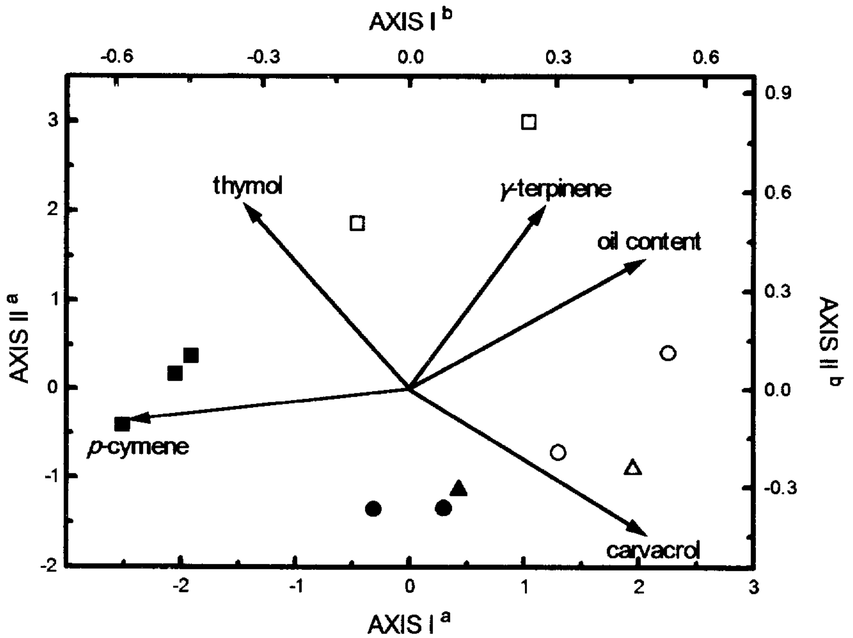

Fig. 2. Principal components analysis (PCA) ordination of *Origanum vulgare* subsp. *hirtum* plants collected from three geographic areas of Greece: Athos (□), Crete (○) and Peloponnese (△) in summer (open symbols) and autumn (solid symbols). [a]Axes refer to the ordination scores obtained for the samples. [b]Axes refer to the ordination scores obtained for the variables which are represented as vectors from the origin. Axis I[a] accounts for the 52.8% of the total variance and Axis II[a] accounts for a further 38.3% of the total variance.

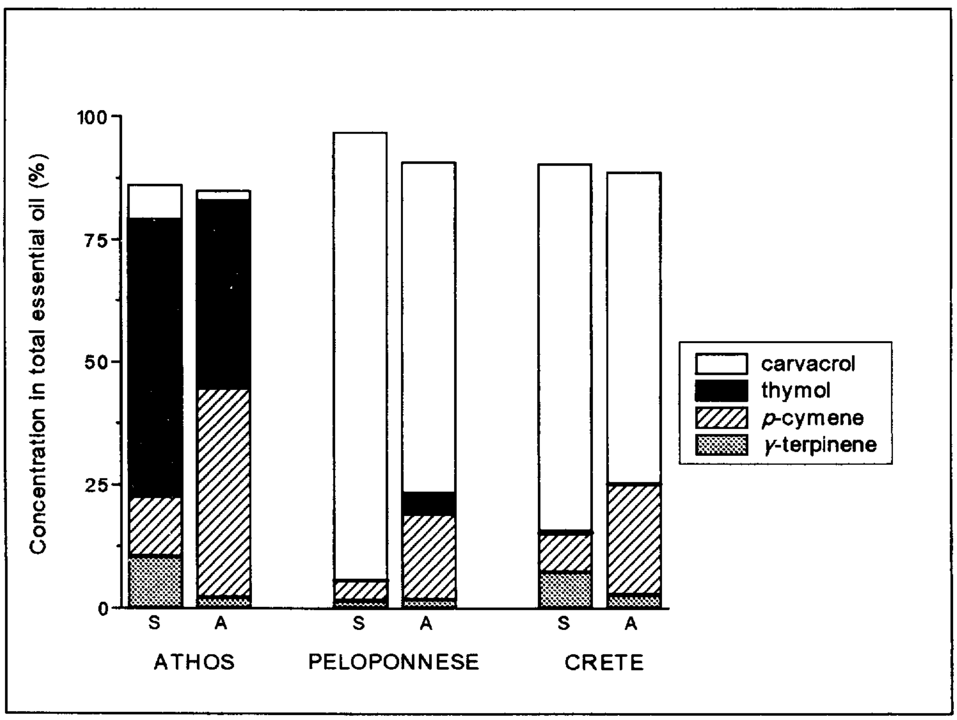

Fig. 3. Concentration (%) of four main components in total essential oil of *Origanum vulgare* subsp. *hirtum* plants collected from three geographic regions of Greece in summer (S) and autumn (A).

hirtum plants collected in autumn from Athos peninsula have not been reported in any oregano or thyme oil. Thus, Greek oregano, which is generally considered as the best oregano spice [12, 13], may be devoid of its characteristic commercially valuable odour when the plants are collected in this period.

In summary, the results of the present study have shown that the essential oils of *O. vulgare* subsp. *hir-*

tum are characterized by stability, irrespective of the season of collection, in respect of the high concentration of the sum of the four components involved in the phenolic biosynthetic pathway and the predominant phenol type. Conversely, the season of collection strongly affects the essential oil yield of the plants (being much lower in autumn) and the concentration of the two phenol precursors; between them *p*-cymeme content is increased in late autumn, being in some cases the major oil constituent. The results suggest that the study of the annual variation pattern will provide more evidence both for the biosynthetic interconversions of essential oil constituents in the different stages of plant development and for the better commercial exploitation of oregano plants.

EXPERIMENTAL

Plant material. O. vulgare subsp. *hirtum* plants were collected in November 1995 from six localities of three geographic areas of Greece (Fig. 1); 25 individuals, representing the local population, were collected from each locality. The inflorescences were dried during the time of collection. Voucher specimens of the collected plants are kept in the Herbarium of the Institute of Systematic Botany and Phytogeography, University of Thessaloniki (TAU). Aerial parts of collected plants were air-dried at room temp. for 10 days. Then, the total plant material from each population was pulverized and submitted to hydrodistillation (2 hr) using a Clevenger apparatus. Essential oil content is expressed in ml 100 g^{-1} dry wt.

GC and GC-MS analyses. GC was carried out with FID, using a 60 m × 0.25 mm Supelcowax 10 capillary column with the following temp. programme. 70° isothermal for 10 min, then an increasing rate of 4° min^{-1} up to 180°, isothermal for 35 min, and then an increasing rate of 4° min^{-1} up to 220°, and isothermal for 5 min. Injector and detector temps were 240°. Carrier gas was He at 0.6 ml min^{-1}. GC-MS analyses were conducted using a system equipped with a capillary column under the same GC conditions. The detector was a quadropolar system with an ionization energy of 70 eV. Individual components were identified by comparing their RR_t and mass spectra with those of the authentic samples, literature [14–15] and a computerized MS-data base [16].

Statistical analysis. Principal component analysis (PCA) [17] was applied to examine the interrelationships between the plants of different geographic areas collected in two seasons (summer and autumn) using the following variables: total essential oil yield, γ-terpinene, *p*-cymeme, thymol, and carvacrol amount in the total essential oil. Summer values used in this analysis and in Fig. 3 were obtained from refs [1–4].

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