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GLANDULAR HAIRS, NON-GLANDULAR HAIRS, AND ESSENTIAL OILS IN THE WINTER AND SUMMER LEAVES OF THE SEASONALLY DIMORPHIC *THYMUS SIBTHORPII* (LAMIACEAE)

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Abstract: The structure and function of the glandular and non-glandular hairs, and also the yield and chemical composition of the essential oils in the winter and summer leaves of the seasonally dimorphic plant *Thymus sibthorpii* were studied. Glandular hairs comprise peltate hairs only (capitate hairs are missing). Peltate hairs are the sites of essential oil biosynthesis. They are more numerous in the winter leaves than in the summer leaves and consist of a 12-celled secretory head, a unicellular stalk, and an also unicellular epidermal foot. The essential oil of the winter leaves is mainly composed of linalool (42.4%), thymol (7.0%), p-cymene (5.8%), β-caryophyllene (5.7%), borneol (5.6%), and terpinen-4-ol (4.8%). The oil of the summer leaves is principally constituted of p-cymene (25.0%), linalool (19.1%), terpinen-4-ol (8.5%) and borneol (8.3%). Non-glandular hairs proliferate in the summer leaves. They are conical in shape and consist of one basal epidermal cell and one apical pointed cell. Glandular and non-glandular hairs are implicated in the chemical and mechanical defense of the plant, respectively.

Keywords: anatomy, leaf hairs, *Thymus sibthorpii*.

Introduction

The aerial organs of many plants are covered with hairs which exhibit a great diversity in shape, size, structure and function. According to WEISS's (1867) definition, plant hairs are structures which owe their origin to outgrowths of single epidermal cells, eventually accompanied by divisions. Similarly, UPHOF (1962) considers that the name "trichome" should be applied to all outgrowths of the epidermis of leaves, shoots and roots, no matter whether they are unicellular or pluricellular. The distinction of plant hairs into "glandular" and "non-glandular" which is largely used today, has its origination to SOLEREDER (1908).

The functional role of plant hairs is multifarious. Non-glandular hairs were found to have an implication in reduction of transpiration and leaf overheating, and also in protection from UV-B radiation [MANETAS, 1999]. Their principal role, however, is mechanical protection against various predators, and particularly the insects (obstacles in insect movement, feeding, and oviposition on leaves) [GOERTZEN & SMALL, 1993]. Glandular hairs, on the other hand, exert chemical protection by secreting different kinds of secondary metabolites which may be repellent and lethal to insects, skin irritant and deleterious to mammals, and toxic to microorganisms [ROSENTHAL & BERENBAUM, 1991; AZAZ & al. 2004].

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The objectives of the present work comprised determination of the types of glandular and non-glandular hairs in *Thymus sibthorpii* and examination of their anatomy, in order to compare them with other *Thymus* species. Moreover, the size, density distribution, and morphometry of the hairs, as well as the chemical constitution of the secreted essential oils were studied in winter and summer leaves in an attempt to find out whether low and high temperatures have any possible effects on these parameters. The obtained data would provide useful information to taxonomists dealing with plant hairs, and also to ecologists working on plant adaptation.

Materials and methods

Plant material and sampling

Native plants of *Thymus sibthorpii* Bentham (Lamiaceae) were studied in the region of Ormylia, Chalkidiki, N. Greece (N 40°16'53", E 23°31'43", altitude 51m a.s.l.). In this region, the meteorological data in the three years of study (2009-2011) showed that during the winter months the average daily air temperature was 7.3 °C, the average daily relative air humidity 78.0%, and the average daily rainfall 1.9 mm. During the summer months, the climatic conditions were mild (not hot and dry) with an average daily air temperature of 24.3 °C, an average daily relative air humidity of 63.3%, and an average daily rainfall of 1.0 mm. Meteorological data were provided by the Regional Center for Plant Protection and Quality Control, Thermi, Thessaloniki, Greece. Winter sampling was performed in January and summer sampling in July. Fully-expanded leaves of annual shoots were used (3rd node from the shoot basis).

Microscopy

From a sample of 18 leaves (3 leaves x 6 plants), 5 leaves were randomly selected for light microscopy (LM), and another 5 leaves for scanning electron microscopy (SEM). Leaves for LM were cut into small pieces which were subsequently fixed for 3h with 5% glutaraldehyde in 0.05 M phosphate buffer (pH 7.2). After washing in buffer, the specimens were post-fixed for 4h with 2% osmium tetroxide, similarly buffered. Samples were then dehydrated in an ethanol series (50-100%) and finally embedded in Spurr's resin. Semithin sections (1 μm thick) of plastid embedded tissue were obtained with a Reichert Om U_2 microtome (Reichert Optische Werke AG, Vienna, Austria), stained with Toluidine Blue O and photographed on a Nikon Eclipse ι80 light microscope (Nikon Instruments, Amstelvee, The Netherlands). For SEM, the specimens, after fixation and dehydration, were critical-point dried in a Balzers CPD 030 device (Balzers Union AG, Liechtenstein) and then carbon-coated in a Jeol JEE-4X vacuum evaporator. Observations were made with a Jeol JSM 840-A scanning electron microscope.

Morphometry

The densities (No/mm^2) of the glandular and non-glandular hairs on both surfaces of the winter and summer leaves were determined using 36 SEM micrographs. These micrographs were also used for conducting morphometric assessments on the hairs.

Essential oils

Leaf material was air-dried at room temperature and then grossly pulverized and subjected to hydrodistillation for 2h using a modified Clevenger-type apparatus. The oil content was expressed in ml/100 g leaf dry weight. Essential oil analyses were performed on a Shimadzu GC-2010-GCMS-QP 2010 system operating at 70eV. This was equipped with a split/splitless injector (230 °C) and a fused silica HP-5MS capillary column (30 m x

0.25 mm i.d., film thickness 0.25 μm). The temperature program ranged from 50 °C to 290 °C, at a rate of 4 °C/min. Helium was used as a carrier gas at a flow rate of 1.0 ml/min. Injection volume of each sample was 1 μl. Arithmetic indices for all compounds were determined using n-alkanes as standards [VAN DEN DOOL & KRATZ, 1963]. Relative percentage of separated compounds was calculated from total ion chromatogram by a computerized integrator. The identification of the components was based on comparison of their mass spectra with those of NIST21 and NIST107 [MASSADA, 1976], and on comparison of their arithmetic indices with literature data [ADAMS, 2007]. Essential oils were often subjected to co-chromatography with authentic compounds (Fluka, Sigma).

Statistics

Statistical analysis was performed with the SPSS package (SPSS Inc. Chicago, USA) using ANOVA for comparison of means between treatments. Significance was determined at $p \leq 0.05$ probability level.

Results

Thymus sibthorpii plants have an entirely different appearance in winter and summer (Fig. 1A, B), a fact principally due to the different environmental conditions prevailing in each of these seasons. Thus, winter plants (Fig. 1A) compared to summer plants (Fig. 1B) are greatly smaller, with shorter densely-arranged shoots and shorter, dark-green leaves. Scanning electron microscopy disclosed that both the winter and summer leaves bear on their surfaces glandular and non-glandular hairs (Fig. 1C). Glandular hairs consist of essential oil-secreting peltate hairs only (Fig. 1C, large asterisks) with no presence of capitate hairs. Peltate hairs are more numerous in the winter leaves (Tab. 1) and they are constructed of a 12-celled secretory head (Fig. 2B), a unicellular stalk and an also unicellular epidermal foot. The 12 head cells are arranged in such a manner that 4 small cells are located in the centre of the head and 8 large ones in the periphery. The foot cell is radially surrounded by 13-15 elongated epidermal cells (Fig. 2C). Non-glandular hairs proliferate in the summer leaves and particularly on their upper side (Tab. 1). They are of one type only, i.e. short conical structures covered with granula (Fig. 1C, small asterisks; Fig. 2D). Their size (height, thickness) does not appear to differ between the winter and summer leaves. Anatomically, non-glandular hairs are composed of one large basal cell located at the level of the epidermis and one pointed cell sited above it (Fig. 2E).

Comparative quantitative analyses of the leaf essential oils in *T. sibthorpii* showed that the winter leaves have a higher essential oil yield (1.20%, i.e. 1.20 ml /100 g leaf d.w.) compared to the summer leaves (1.11%) (Tab. 2). Qualitative analyses of the winter and summer oils disclosed the existence of 49 compounds accounting for 97.6-99.3% of the total oils (Tab. 2). Both types of oils contain as principal components p-cymene, linalool, borneol, terpinen-4-ol, thymol, and β-caryophyllene. The major constituent of the winter oil is linalool (42.4%), followed by thymol (7.0%), p-cymene (5.8%), β-caryophyllene (5.7%), borneol (5.6%) and terpinen-4-ol (4.8%). The major constituent of the summer oil is p-cymene (25.0%), followed by linalool (19.1%), terpinen-4-ol (8.5%) and borneol (8.3%). Thymol and β-caryophyllene occur at a low percentage (about 1%).

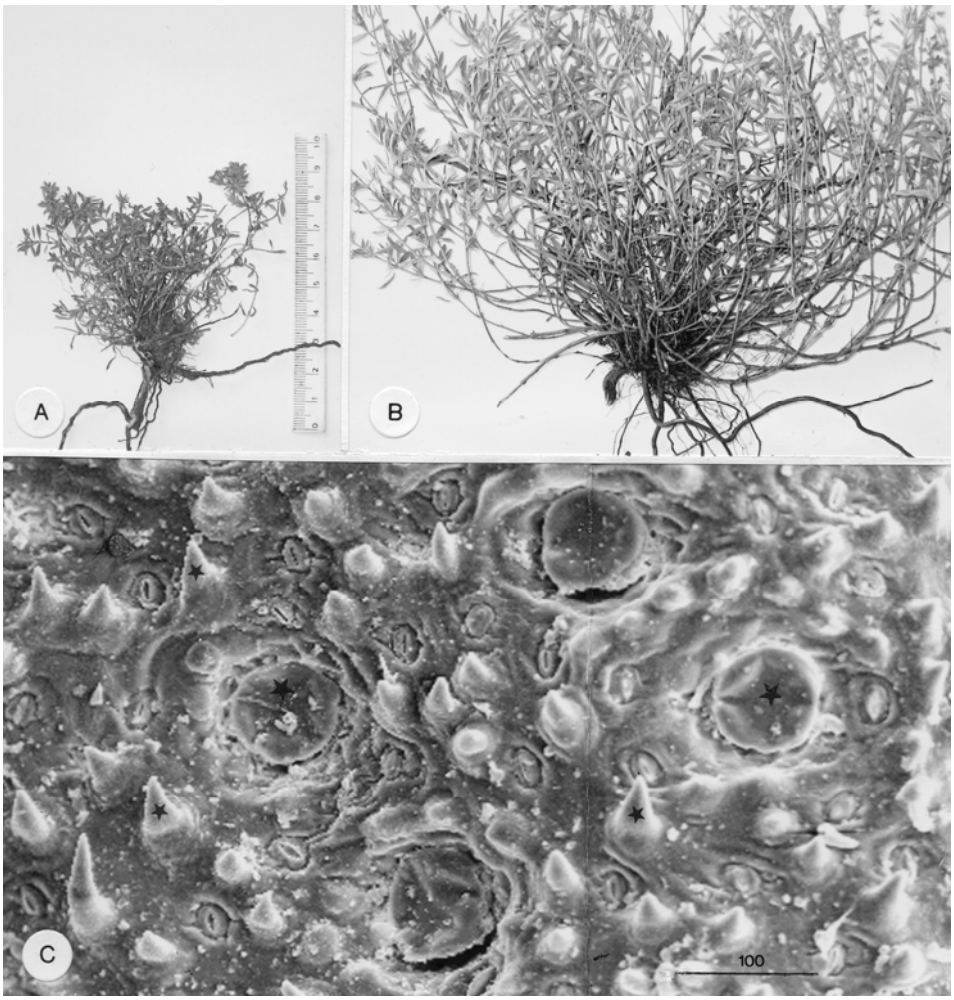

Fig. 1. *Thymus sibthorpii*. Lateral view of herbarium material of winter plant (A) and summer plant (B). Compare the size and density of shoots and leaves. C. SEM view of the leaf surface with peltate glandular hairs (large asterisks) and conical non-glandular hairs (small asterisks). Bar in μm.

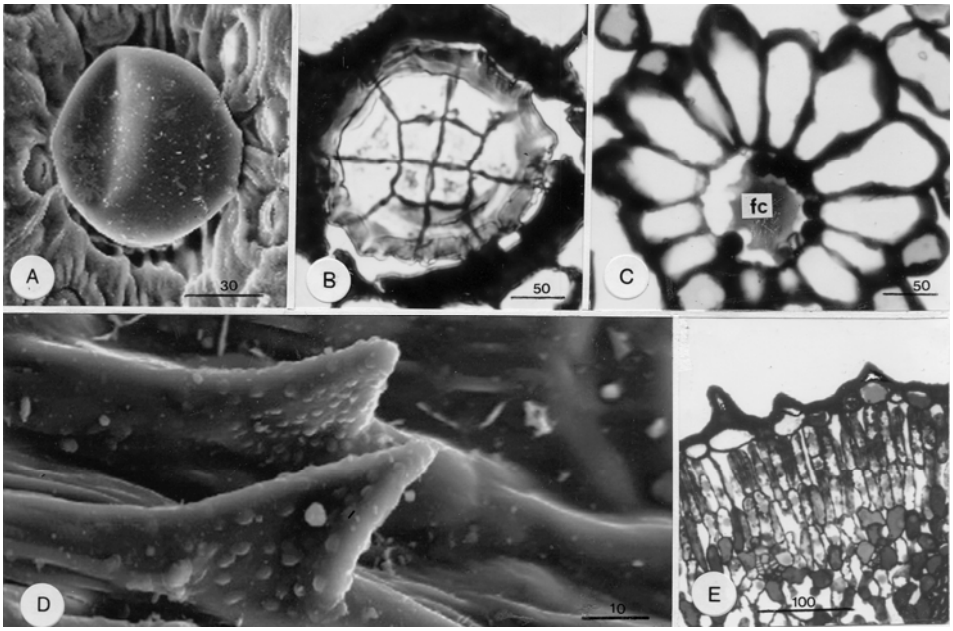

Fig. 2. A. SEM view of a peltate hair. B. Paradermal section of the head of a peltate hair. The head consists of 12 cells. C. Paradermal section of the foot cell (fc) which appears radially surrounded by 13-15 elongated epidermal cells. D. Non-glandular hairs as they appear in SEM. E. Longitudinal section of non-glandular hairs composed of a large basal epidermal cell and a small apical pointed cell. Bars in μm.

Discussions

SEM observations on the blade surfaces of the winter and summer leaves of *Thymus sibthorpii* revealed the presence of numerous peltate glandular hairs and the absence of capitate glandular hairs. SATIL & al. (2005) also reported a rare presence of capitate hairs in the leaves of *T. migricus.* However, studies on *T. malyi, T. vulgaris* and *T. capitatus* mentioned the existence of capitate glandular hairs with a unicellular head [WERKER & al. 1985; MARIN & al. 2008; BOZ & al. 2009]. The peltate hairs in *T. sibthorpii* appeared to consist of a secretory head with 12 cells. The number of head cells in the peltate hairs of other *Thymus* species was found to fluctuate, as in *T. capitatus* where the head consisted of 14 cells [WERKER & al. 1985], in *T. serpyllum* of 8 cells [UPHOF, 1962], in *T. vulgaris* of 8-12 cells [YAMAURA & al. 1992] and also of 10-14 cells [BRUNI & MODENESI, 1983], and in *T. malyi* of 8 cells [MARIN & al. 2008]. The peltate hairs and specifically their head cells are the only cells from all leaf tissues which possess the necessary enzymic equipment for essential oil biosynthesis [McCASKILL & al. 1992]. Thus, the higher the number of peltate hairs on leaves is, the higher the amount of the essential oil derived by distillation. This is in accordance with the morphometric assessments on *T. sibthorpii* in which the higher number of peltate hairs on winter leaves compared to summer leaves resulted in a higher essential oil yield.

The elongated epidermal cells which radially surround the foot cell of each peltate hair (peribasal cells) are considered as a fundamental accessory of the hair. Thus, the large surface area defined by the radial arrangement of the peribasal cells, their convergence towards the foot cell, and their location at the border with the mesophyllic photosynthetic parenchyma, favour the interpretation that peribasal cells collect from the mesophyll photosynthates which become centripetally transferred first to the foot cell (large central vacuole) and then to the apical head cells. There, they will constitute the precursors for the biosynthesis of the essential oil.

Qualitative analysis of the essential oils from *T. sibthorpii* winter and summer leaves showed that in both types of oils the major components are linalool and p-cymene. Linalool dominated in the winter leaves and p-cymene in the summer leaves. The high content in linalool of the thyme oil is ascribed by VERNET & al. (1986) to the low environmental temperatures (winter conditions). In accordance to our results, high contents of p-cymene, γ-terpinene, borneol, and terpinen-4-ol were also recorded by VOKOU (1993) in the oil of the summer leaves of *T. capitatus.*

Tab. 1. *Thymus sibthorpii.* Morphometric assessments of peltate glandular hairs and conical non-glandular hairs in fully expanded leaves of winter and summer (±SD, n=36). Means between columns with different letters are significantly different at 0.05 level

	Winter leaves	Summer leaves
Density of peltate glandular hairs on the upper leaf side (No/mm^2)	20.7±2.9 a	12.8±2.1 b
Density of peltate glandular hairs on the lower leaf side (No/mm^2)	8.8±0.3 a	7.7±0.4 b
Head diameter of peltate glandular hairs in surface view on the upper leaf side (μm)	75.9±6.5 a	85.4±9.1 b
Head diameter of peltate glandular hairs in surface view on the lower leaf side (μm)	71.7±5.7 a	82.6±8.5 b
Density of conical non-glandular hairs on the upper leaf side (No/mm^2)	149.6±17.6 a	164.5±19.3 b
Density of conical non-glandular hairs on the lower leaf side (No/mm^2)	*	62.6±5.3
Thickness of the base of conical non-glandular hairs on the upper leaf side (μm)	26.5±2.3 a	27.1 ±3.0 a

Thickness of the base of conical non-glandular hairs on the lower leaf side (μm)	*	10.1±2.2
Height of conical non-glandular hairs on the upper leaf side (μm)	39.8 ±5.7 a	40.2±8.6 a
Height of conical non-glandular hairs on the lower leaf side (μm)	*	18.3±7.8

*There are no non-glandular hairs on the lower leaf side of the winter leaves

Tab. 2. *Thymus sibthorpii*. Qualitative and quantitative compositions (%) of the essential oils from winter and summer leaves

Components [a]	AI [b]	Winter leaves	Summer leaves	Identification [c]
α-Thujene	926	n.d.	0.4	AI, MS
α-Pinene	932	n.d.	1.0	AI, MS, Co-GC
Camphene	946	0.2	1.7	AI, MS
Sabinene	972	n.d.	0.2	AI, MS
β-Pinene	974	n.d.	0.3	AI, MS, Co-GC
1-Octen-3-ol	980	1.0	0.3	AI, MS
3-Octanone	988	0.5	0.4	AI, MS
β-Myrcene	991	0.1	0.2	AI, MS, Co-GC
3-Octanol	997	0.6	0.2	AI, MS
α-Phellandrene	1002	n.d.	0.3	AI, MS
α-Terpinene	1017	0.5	1.7	AI, MS
***p*-Cymene**	**1024**	**5.8**	**25.0**	**AI, MS, Co-GC**
Limonene	1028	0.5	4.5	AI, MS, Co-GC
1,8-Cineole	1029	1.1	4.4	AI, MS, Co-GC
γ-Terpinene	1058	2.4	3.6	AI, MS, Co-GC
cis-Sabinene hydrate	1067	0.5	3.2	AI, MS
cis-Linalool oxide (furanoid)	1072	0.3	0.5	AI, MS
Terpinolene	1087	n.d.	0.8	AI, MS
trans-Linalool oxide (furanoid)	1087	0.6	0.5	AI, MS
trans-Sabinene hydrate	1095	n.d.	0.5	AI, MS
Linalool	**1100**	**42.4**	**19.1**	**AI, MS, Co-GC**
cis-Verbenol	1138	0.1	n.d.	AI, MS
trans-p-Menth-2-en-1-ol	1138	0.1	n.d.	AI, MS
Camphor	1142	0.5	1.0	AI, MS, Co-GC
Borneol	**1165**	**5.6**	**8.3**	**AI, MS, Co-GC**
Terpinen-4-ol	**1176**	**4.8**	**8.5**	**AI, MS, Co-GC**
p-Cymen-8-ol	1184	0.2	0.4	AI, MS

α-Terpineol	1190	0.3	0.8	AI, MS
cis-Dihydro carvone	1196	0.1	0.3	AI, MS
trans-Dihydro carvone	1203	0.2	0.3	AI, MS
trans-Carveol	1219	0.1	0.3	AI, MS
Cumin aldehyde	1240	n.d.	0.1	AI, MS
Carvone	1244	n.d.	0.1	AI, MS, Co-GC
Carvacrol methyl ether	1244	n.d.	0.1	AI, MS
Bornyl acetate	1288	0.2	0.2	AI, MS, Co-GC
Thymol	**1292**	**7.0**	**1.0**	**AI, MS, Co-GC**
Carvacrol	1301	3.9	2.3	AI, MS
Thymol acetate	1355	0.2	n.d.	AI, MS
Carvacrol acetate	1374	0.1	n.d.	AI, MS
β-Bourbonene	1384	0.7	0.5	AI, MS
β-Caryophyllene	**1421**	**5.7**	**1.4**	**AI, MS, Co-GC**
cis-Cadina-1(6),4-diene	1462	0.2	n.d.	AI, MS
9-epi-(E)-Caryophyllene	1462	0.2	n.d.	AI, MS
Germacrene D	1482	2.1	n.d.	AI, MS
Bicyclogermacrene	1497	2.6	n.d.	RI, MS
β-Bisabolene	1509	3.8	0.7	AI, MS
Spathulenol	1578	0.7	0.4	AI, MS
Caryophyllene oxide	1583	3.3	2.1	AI, MS, Co-GC
Caryophylla-4(12), 8(13)-dien-5-ol [d]	1639	0.1	n.d.	AI, MS
TOTAL		**99.3**	**97.6**	
Essential oil yield (%)		**1.20**	**1.11**	

[a] Compounds listed in order of elution from an HP-5 MS capillary column; [b] AI: Arithmetic indices as determined on a HP-5 MS capillary column using a homologous series of n-alkanes (C9-C25); [c] Identification method: AI= Arithmetic index. MS=mass spectrum. Co-GC=coinjection with authentic compound. [d] Correct isomer not identified. n.d.: not detected

Conclusions

Thymus sibthorpii plants bear on their leaves glandular and non-glandular hairs which differ in anatomy from other *Thymus* species. The structural model of the glandular hairs (peribasal cells, basal cell, head cells) is functionally related to the uptake of photosynthates from the mesophyll and their transportation to the head cells where they constitute precursors for the biosynthesis of the essential oil. In winter leaves, glandular hairs are more numerous and secrete a higher amount of essential oil, compared to summer leaves. Due to the antioxidant properties of the essential oils, the plant can thus tolerate winter low temperatures which stimulate oxidative stress.

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