

Glandular and non-glandular hairs in the winter and summer leaves of the seasonally dimorphic *Teucrium polium* (Lamiaceae)

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Received: 24. September 2013 / Accepted: 02. November 2013 / Available online: 03. November 2013 / Printed: December 2013

Abstract. *Teucrium polium* is a seasonally dimorphic plant having short shoots and leaves in winter and long shoots and leaves in summer. Both the winter and summer leaves are covered with an indumentum of glandular and non-glandular hairs. Winter leaves bear more numerous hairs compared to summer leaves. Glandular hairs comprise peltate and capitate hairs. Peltate hairs consist of a unicellular epidermal foot, an also unicellular stalk, and a 4-celled head. Capitate hairs are of two sorts, long and short. Long hairs are composed of a unicellular epidermal foot, a 3-celled stalk, and a unicellular head, while short hairs of a unicellular foot, a unicellular stalk, and a 2-celled head. Histochemical tests showed that peltate hairs secrete essential oil and capitate hairs mucilage. Non-glandular hairs are mostly dendroid multicellular, but uniseriate multicellular hairs also exist. Both glandular and non-glandular hairs are implicated in chemical and mechanical defence against winter cold stress.

Keywords: Seasonal dimorphism, glandular hairs, non-glandular hairs, *Teucrium polium*.

Introduction

Teucrium polium is an evergreen perennial subshrub growing wildly at altitudes up to 2000 m. The leaves on the shoots follow a decussate phylotaxis and the flowers are arranged in apical inflorescences (racemes). The genus *Teucrium* is a large genus with about 340 species distributed in almost all continents (temperate regions), but particularly in the southern part of Europe (Brown 1995, Navaro & El Qualidi 2000). Many *Teucrium* species have potent biological activities against diabetes, inflammation, sepsis, hypertension, ulcer, and cancer, and they also have antimicrobial and antioxidant properties (Brown 1995, Kandouz et al. 2010).

T. polium is a typical seasonally dimorphic plant with entirely different appearance in winter and summer. In the study area, the plant undergoes in winter a low temperature stress (oxidative stress) and reacts by developing chemical and mechanical defence through glandular and non-glandular hairs. Glandular hairs produce essential oils with antioxidant activities while non-glandular hairs create on the leaf surface a thick insulating layer which prevents penetration of the cold into the mesophyll. Under this regard, we studied the leaf indumentum of *T. polium*, providing in parallel information to taxonomists dealing with application of plant trichomes to modern systematics.

Materials and methods

Plant material and sampling

Teucrium polium L. (Lamiaceae) was studied in the region of Ormylia, Chalkidiki, N. Greece (N 40°16'53", E 23°31'44", altitude 48 m a.s.l.). In this region, the meteorological data in the three years of study (2009, 2010, 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 condition were mild (not hot and dry) with an average daily temperature of 24.3°C, and average daily relative air humidity of 63.3%, and an average daily rainfall of 1 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 August. Fully-expanded leaves of annual shoots were used (3rd node from the shoot basis).

Microscopy (LM, SEM)

A random sample of 10 leaves was used for light microscopy (LM) and scanning electron microscopy (SEM) separately for winter and summer. Pieces of leaves for LM were pre-fixed for 3h with 5% glutaraldehyde in 0.05 M phosphate buffer (pH 7.2) and post-fixed for 4h with 2% osmium tetroxide, similarly buffered. Samples were then dehydrated in an alcohol series (50-100%) and finally embedded in Spurr's resin. Semithin sections (1 µm thick) for LM were obtained with a Reichert Om U₂ microtome (Reichert Optische Werke AG, Vienna, Austria), stained with Toluidine Blue O, and photographed on a Nikon Eclipse i80 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 density of glandular and non-glandular hairs on both leaf surfaces and their size parameters were assessed using 36 SEM micrographs (x 160).

Histochemistry

Leaf paradermal free-hand sections were stained with 2% aqueous osmium tetroxide for identification of lipidic substances. Microtome semithin sections (1 µm thick) of plastic embedded tissue were stained with Periodic Acid-Schiff reagent (PAS) for identification of acidic polysaccharides (Nevalainen et al. 1972). Semithin sections were also stained with 1% Coomassie Brilliant Blue in 7% acetic acid for identification of proteins (Fisher 1968).

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

Teucrium polium is a characteristic seasonally dimorphic plant with diverging morphology in winter and summer (Fig. 1). Winter plants are hamaephytes with short shoots (about 7 cm in length) arranged in a cluster (Fig. 1A). They bear small leaves (average surface area per leaf size of 9.2 mm²) which undergo rolling (Fig. 2A). Summer plants, on the other hand, are much taller reaching a length of 27 cm



Figure 1. *Teucrium polium*. Herbarium material from winter plant (A) and summer plant (B).

Table 1. *Teucrium polium*. Morphometric assessments of peltate glandular hairs and dendroid non-glandular hairs in fully expanded leaves of winter and summer (\pm 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)	48.5 \pm 7.2 a	39.5 \pm 4.9 b
Density of peltate glandular hairs on the lower leaf side (No/mm ²)	22.4 \pm 1.8 a	6.7 \pm 0.4 b
Head diameter of peltate glandular hairs in surface view on the upper leaf side (μ m)	53.3 \pm 4.7 a	53.4 \pm 2.3 a
Head diameter of peltate glandular hairs in surface view on the lower leaf side (μ m)	52.1 \pm 6.2 a	52.8 \pm 5.8 a
Density of dendroid non-glandular hairs on the upper leaf side (No/mm ²)	163.6 \pm 11.8 a	240.6 \pm 23.6 b
Density of dendroid non-glandular hairs on the lower leaf side (No/mm ²)	98.6 \pm 8.3 a	63.4 \pm 5.5 b
Thickness of the stem of dendroid non-glandular hairs on the upper leaf side (μ m)	13.7 \pm 2.7 a	14.1 \pm 3.1 a
Thickness of the stem of dendroid non-glandular hairs on the lower leaf side (μ m)	14.3 \pm 3.6 a	14.8 \pm 2.5 a
Height of the stem of dendroid non-glandular hairs on the upper leaf side (μ m)	81.3 \pm 9.9 a	82.1 \pm 7.0 a
Height of the stem of dendroid non-glandular hairs on the lower leaf side (μ m)	82.2 \pm 11.1 a	82.4 \pm 7.3 a

(including the apical inflorescences) (Fig. 1B). Their leaves have increased size exhibiting an average surface area per leaf side of 89.9 mm². Both the winter and summer leaves are covered with a dense indumentum of glandular and non-glandular hairs (Figs 2A, 2B, 2C). Glandular hairs proliferate in winter leaves (on both leaf sides) compared to summer leaves (Table 1). They do not appear to differ in size in the two sorts of leaves. Non-glandular hairs are more numerous on the upper side of the summer leaves and the lower side of the winter leaves (Table 1). Their size parameters do not significantly differ between the winter and summer leaves.

Glandular and non-glandular hairs are easily discernible on the leaf surface of *T. polium* by scanning electron microscopy. Glandular hairs consist of peltate and capitate hairs (Fig. 2C; p, c) while non-glandular hairs of elongated filamentous structures forming a network (Fig. 2C, asterisks). Peltate glandular hairs are implicated in essential oil secretion. They are composed of a 4-celled head (Fig. 3A), a unicellular stalk, and an also unicellular epidermal foot (Fig.

3B). At the apex of the head a bladder is formed (by raising of the cuticle) containing the essential oil (Fig. 3B). The lipophilic nature of the essential oil in the apical bladder is evidenced after treatment with the lipophilic osmium tetroxide which stained the oil black (Fig. 3C). Capitate glandular hairs are of two types, long and short. Long capitate hairs consist of a unicellular large epidermal foot, a 3-celled stalk of 2 long cells and 1 short apical cell, and a unicellular globular head (Fig. 3D). Short capitate hairs consist of a unicellular epidermal foot, a unicellular stalk, and a 2-celled head (Fig. 3G). Histochemical tests with the PAS reagent, specific for acidic polysaccharide identification, showed that the secretory head of the long and also of the short capitate hairs became heavily stained pink/purple (Figs 3E, 3H). Application of the dye Coomassie Brilliant Blue for identification of proteins, further showed a deep blue staining of the secretory head of the long and the short capitate hairs as well (Figs 3F, 3I).

Non-glandular hairs are similarly of two types, i.e. mul-

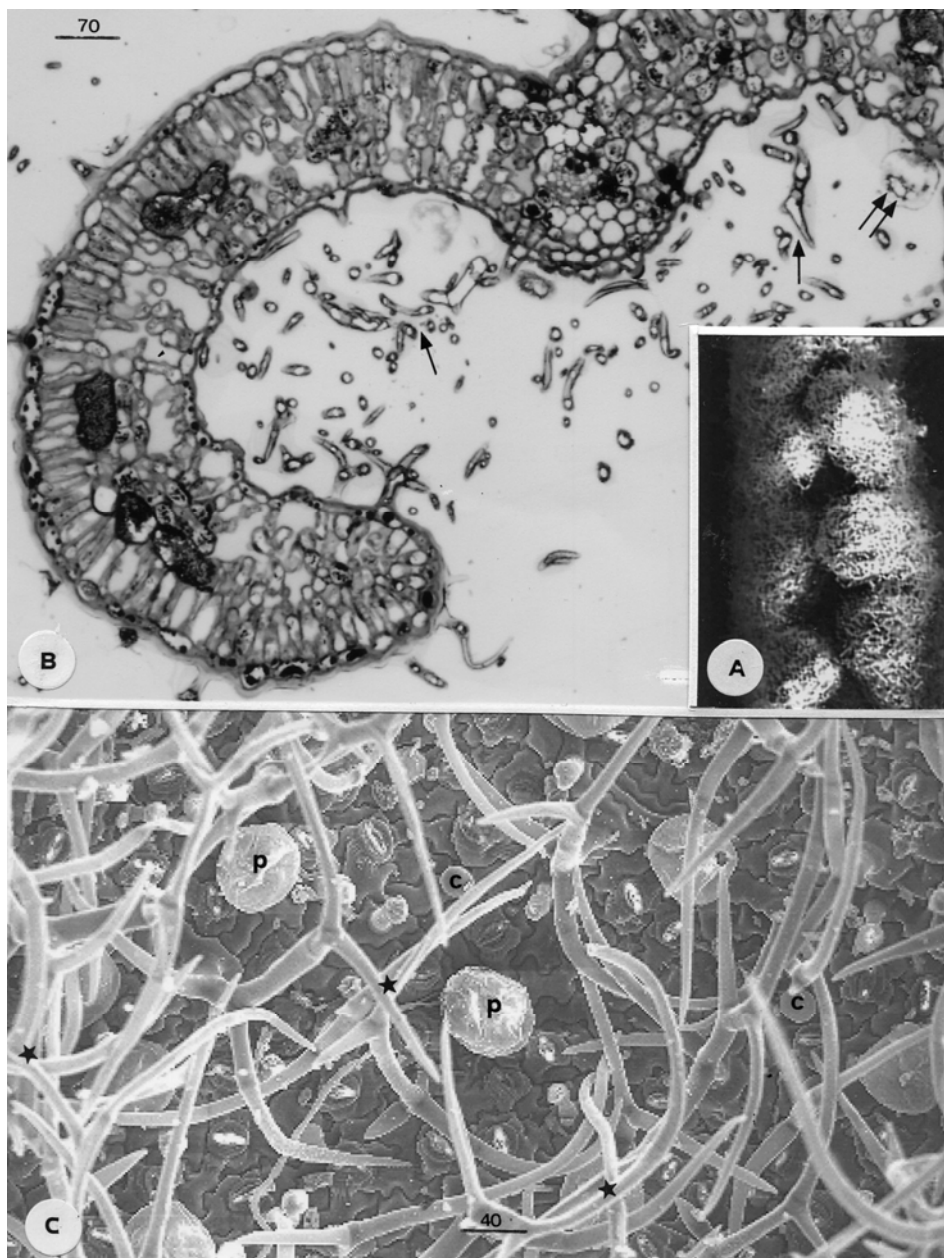


Figure 2. A. A rolled leaf viewed from the lower side. B. Cross-section of a rolled leaf. The profiles of the sectioned non-glandular hairs can be discerned. C. SEM view of the leaf surface with peltate (p) and capitate (c) glandular hairs, and also with elaborating branches of dendroid non-glandular hairs (asterisks). Bars in μm .

ticellular dendroid and multicellular uniseriate (Fig. 4). Multicellular dendroid hairs consist of a vertical stem (82 μm high and 14 μm thick) ordinarily composed of 3 elongated cells (Table 1). The upper part of each stem member-cell extends laterally to form 1-3 unicellular projections (Figs 4A, 4B). Multicellular uniseriate hairs consist of a series of cell-members, the apical one of which is pointed (Fig. 4A, inset). The population of dendroid hairs is largely higher than that of the uniseriate hairs.

Discussion

The leaf glandular hairs we studied in *Teucrium polium* from Greece comprise peltate and capitate hairs. Peltate hairs con-

sist of a 4-celled head, a unicellular stalk, and a unicellular foot. A 4-celled head of peltate hairs has been also reported in *T. polium* from Iran (Eshratifar et al. 2011) and a 4 to 8-celled head in *T. polium* from Croatia (Jurisic Grubescic et al. 2007). In *T. polium* from Serbia/Montenegro, the head of the peltate hairs was mentioned to be just multicellular (Lakusic et al. 2010). Other *Teucrium* species having peltate hairs with a 4-celled head are *T. marum*, *T. flavum*, *T. subspinosum*, *T. scorodonia*, and *T. siculum* (Bini Maleci & Servettaz 1991, Servettaz et al. 1994).

Capitate glandular hairs in the studied *T. polium* are of two types, i.e. long hairs and short hairs. Long capitate hairs occur more frequently than short ones. Published studies on *T. polium* are referred to the presence of either long capitate hairs (Christodoulakis et al. 2010) or short capitate hairs

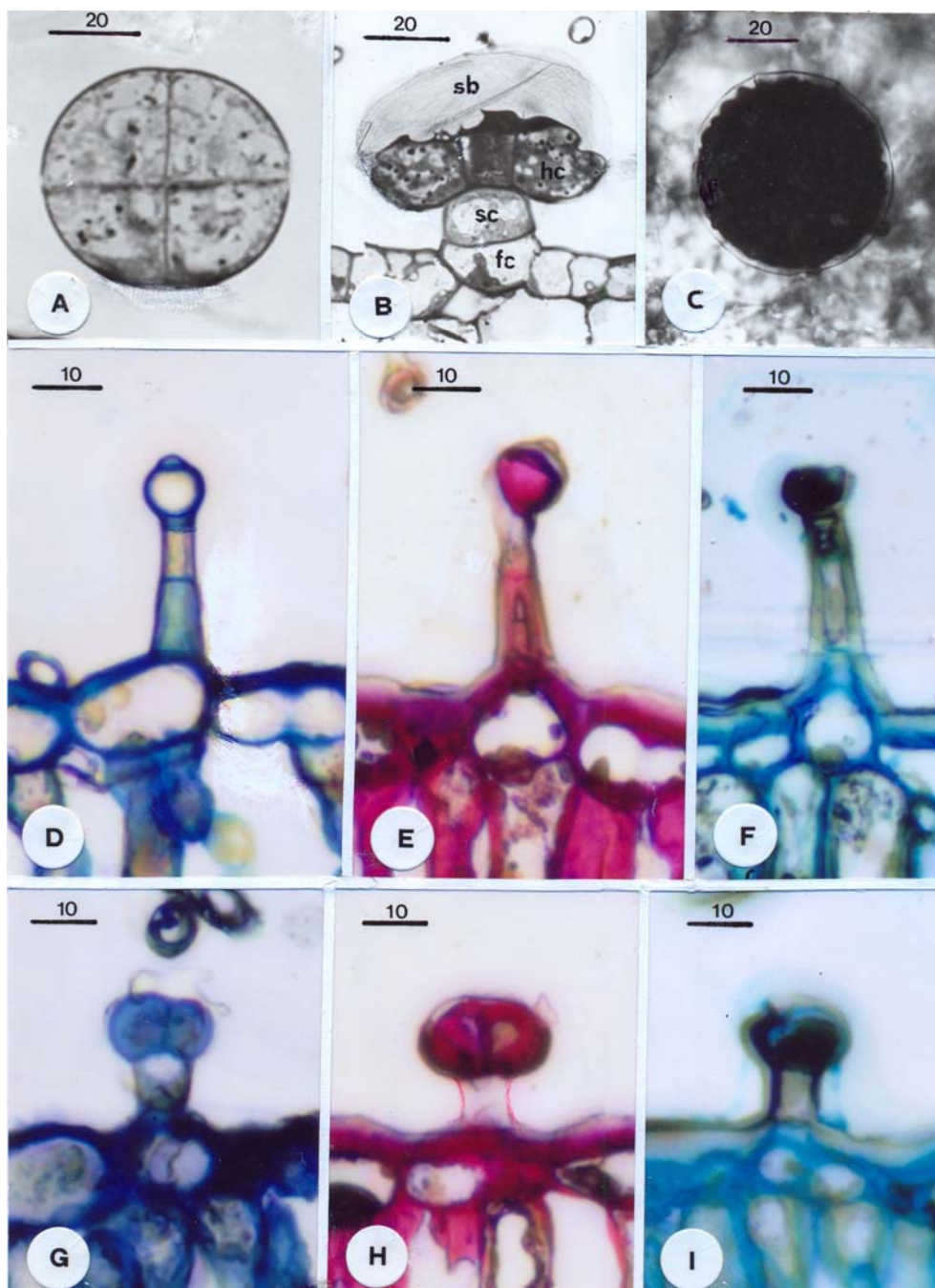


Figure 3. Glandular hairs. A. The head of a peltate glandular hair in leaf paradermal section. It consists of 4 cells. B. Longitudinal section of a peltate hair to show the foot cell (fc), the stalk cell (sc), the head cells (hc), and the subcuticular bladder (sb) filled with essential oil. C. A peltate hair in leaf surface view. The essential oil in the subcuticular bladder is stained black after treatment with the lipophilic osmium tetroxide. D-F. Long capitate glandular hairs stained with Toluidine Blue (general stain) (D), with PAS for acidic polysaccharides (purple colour of the head) (E) and with Coomassie Brilliant Blue for proteins (dark blue colour of the head) (F). G-I. Short capitate glandular hairs stained with Toluidine Blue (G), PAS (H), and Coomassie Brilliant Blue (I). In the latter two cases, the heads of the hairs became heavily stained purple and dark blue, respectively. Bars in μm .

(Jurisic Grubescic et al. 2007), not of both of them. In *T. capitatum*, only long capitate hairs were observed (Antunes et al. 2004) while in *T. marum* and *T. subspinosum* (Servettaz et al. 1992) and also in *T. siculum* and *T. scorodonia* (Servettaz et al. 1994), only short capitate hairs. Peltate glandular hairs appeared in our material to be implicated in secretion of essential oil and capitate glandular hairs in secretion of glycoproteins (mucilage), as histochemical tests showed.

The non-glandular hairs we studied in *T. polium* were of two types, i.e. multicellular dendroid (branched) and multicellular uniseriate. A mixed population of dendroid and uniseriate non-glandular hairs has been also observed in *T. polium* from Croatia and Serbia/Montenegro (Jurisic Grubescic et al. 2007, Lakusic et al. 2010). In some cases, *T. polium* leaves were described to possess only dendroid non-glandular hairs (Christodoulakis et al. 2010, Dinc et al. 2011,

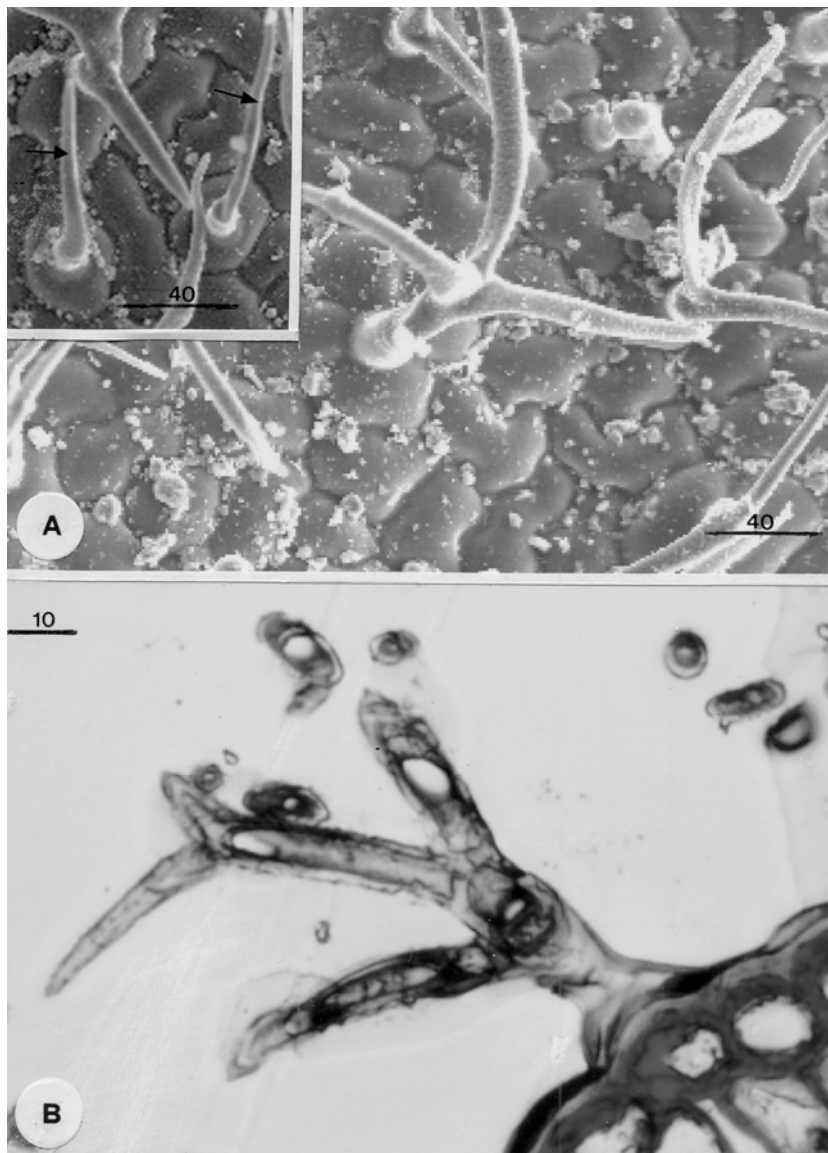


Figure 4. Non-glandular hairs. A. SEM view of a dendroid multicellular hair with 3 branches. 4A, inset. Two uniseriate multicellular hairs (arrows). B. Longitudinal section of a dendroid hair illustrating the stem and the unicellular branches. Bars in µm.

Eshratifar et al. 2011), whereas in other *Teucrium* species (*T. marum*, *T. subspinosum*, *T. botrys*, *T. hamaedrys*, *T. lucidum*, *T. flavum*, *T. scordonia*, *T. siculum*, *T. montanum*, *T. arduini*, *T. scordium*, *T. hircanicum*, *T. parviflorum*, *T. oliverianum*, *T. orientale*) leaves appeared to bear only uniseriate hairs (Bini Maleci & Servettaz 1991, Servettaz et al. 1992, Servettaz et al. 1994, Jurisic Grubescic et al. 2007, Lakusic et al. 2010, Dinc et al. 2011, Eshratifar et al. 2011). The above data reveal that *Teucrium* species exhibit a remarkable diversity as concerns the types of leaf glandular and non-glandular hairs, a fact apparently associated with genetic and environmental factors.

The alterations in density of the glandular and non-glandular hairs on the winter and summer leaves of *T. polium* reflect the response of the plant to the different environmental conditions prevailing in winter and summer. The meteorological data in the study area revealed that weather conditions in summer were mild (do not result in heat or drought stress), whereas in winter, low temperatures acti-

vate oxidative stress (Imahori et al. 2008) which inhibits growth of shoots and leaves (short shoots and leaves). Low temperatures additionally lead to rolling of leaves (to trap warmth inside) and their covering with a thick insulating layer of non-glandular hairs (to prevent penetration of the cold into the mesophyll). Oxidative stress induces an increase in number of the glandular peltate hairs, a fact entailing an increase of the amount of the produced essential oil which has antioxidant properties (Tepe et al. 2011). The glycoproteins (mucilage) secreted by the capitate glandular hairs might have a function as lubricants to the rolling of winter leaves (Kristen 1974).

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