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Anatomical and histochemical investigation of the leaf of *Teucrium polium*, a pharmaceutical sub-shrub of the Greek phryganic formations

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Although extensively studied for its pharmaceutical properties, *Teucrium polium* has yet to disclose its leaf structure and the chemical nature of the accumulated or secreted products. Therefore, light, scanning, and transmission electron microscopy along with histochemical tests were employed to investigate the leaf of this perennial dwarf Mediterranean shrub. Structural characteristics include large cells with cutinized walls, creating cavities of obscured function in contact to a vessel element of the conductive tissue and different types of glandular hairs. A variety of secreted materials was identified by certain histochemical stains within the mesophyll cells and the glandular hairs. A dense indumentum of non-glandular hairs protects the secretory apparatus and the leaf surface from the stressful conditions of the Mediterranean climate.

Key words: *Teucrium polium*, leaf anatomy, histochemistry, secretory structures, secondary metabolites.

INTRODUCTION

It is well documented that more than 11000 plant taxa grow wild in the European continent. Among them, more than 5500 thrive in Greece (Flora Europaea, 1999). About 250 of them are considered to be of great pharmaceutical importance and most were referred and used for medical purposes since the ancient times (Dioscurides, 77 AD), although their use diminished through the centuries. *Teucrium polium* (according to ESFEDS Edinburgh, export date: May 11, 1996. Nomencl. Ref.: *Sp. Pl. 566 (1753)* ©1996-2007 The International Organization for Plant Information. Page updated: August 21, 2007) is a mediterranean dwarf shrub expanding to southern Europe, including the Greek coastland and the islands. It is a phryganic species (Orshan, 1989), traditionally used in folk medicine, like most of the 340 species of the cosmopolitan genus *Teucrium* (Lamiaceae) (Brown, 1995). It is well adapted to the stressful conditions that characterize the mediterranean climate and pose serious challenges for the plants thriving in areas with this type of climate (Mitrakos, 1980). The phryganic sub-shrubs comprise a type of vegetation thriving in the hot and arid boundary of the mediterranean climate (Mooney & Parsons, 1973; Parsons, 1976). They are considered – along with the evergreen sclerophyllous shrubs – as well adapted xerophytes, exhibiting interesting xeromorphic characteristics (Fahn & Cutler, 1992).

After the global demand to shift to natural food additives and cosmetics, plant products regained high interest (Ascensão *et al*., 1999; Corsi & Bottega, 1999; Sacchetti *et al*., 1999). As a result, food and pharmaceutical industries started searching natural sources for active compounds, thus xerophytes began receiving new interest mainly due to their potential for producing secondary metabolites. This production is considered to be a major response of many plant species to the stressful conditions of the mediterranean climate (Fahn, 1988; Christodoulakis & Bazos, 1990; Christodoulakis *et al*., 1990; Christodoulakis & Fasseas, 1991).

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A bulk of literature is available on essential oils, pharmaceutical properties, and the effects of herbal remedies containing *Teucrium polium* (Starakis *et al*., 2006; Ardestani *et al*., 2008; Sharififar *et al*., 2009) and *Teucrium capitatum* (Dourakis *et al*., 2002). Some anatomical data, rather insufficient, are given in papers dealing with other species of the genus *Teucrium* (Dinç *et al*., 2008, 2009) and only a few pieces of insufficient information (Antunes *et al.,* 2004) or in the form of unpublished, personal observations (Fahn & Cutler, 1992) are available on the structural characters and secreting activity of the leaves of *T. capitatum*. Yet, the report of foliar idioblasts in a species of the family Lamiaceae, similar in structure to *T. polium* (Lersten & Curtis, 1998), is of particular interest.

Based on our knowledge on the leaf structure, function, and adaptations of the evergreen sclerophylls (Kummerow, 1973; Christodoulakis & Mitrakos, 1987; Rhizopoulou & Mitrakos, 1989; Christodoulakis, 1992), the seasonally dimorphics (Christodoulakis, 1989; Nikolakaki & Christodoulakis, 2007), and some other typical xerophytic shrubs of Greece (Nikolakaki & Christodoulakis, 2004, 2006) we investigated –by light and electron microscopy (scanning and transmission) as well as with histochemical tests– the leaf of *Teucrium polium* and revealed its xeromorphic character, secretory activity, and adaptation.

MATERIALS AND METHODS

Light and electron microscopy (scanning and transmission)

Mature leaves of *T. polium* growing wild in a practically non-polluted, natural formation east of Athens metropolitan area (37° 54′ N, 23° 45′ E, 350 m elevation), were collected in early (May) and late (September) summer of 2008. They were cut into pieces (1×1 mm) and fixed in phosphate buffered 3% glutaraldehyde (pH 6.8) at 0°C for 2 hrs (Sabatini *et al*., 1963). Some of the leaf segments were dehydrated in a graded acetone series, critical point dried, coated with gold, and observed and photographed with a JEOL JSM-6360 high vacuum scanning electron microscope (SEM) at 20 kV.

The rest of the tissue was post-fixed in 1% osmium tetroxide in phosphate buffer (Ledbetter & Porter, 1963), dehydrated in a graded acetone series, and embedded in Durcupan ACM epoxy resin (Fluka, Steinheim, Switzerland). For the observation of the uranyl acetate-lead citrate double-stained ultra-thin sections (Reynolds, 1963) a JEOL 100S Transmission Electron Microscope was used. Micrographs were recorded with an OLYMPUS Megaview G2 digital camera. Semi-thin and ultra-thin sections were obtained on an LKB (Sweden) Ultrotome III.

Histochemistry

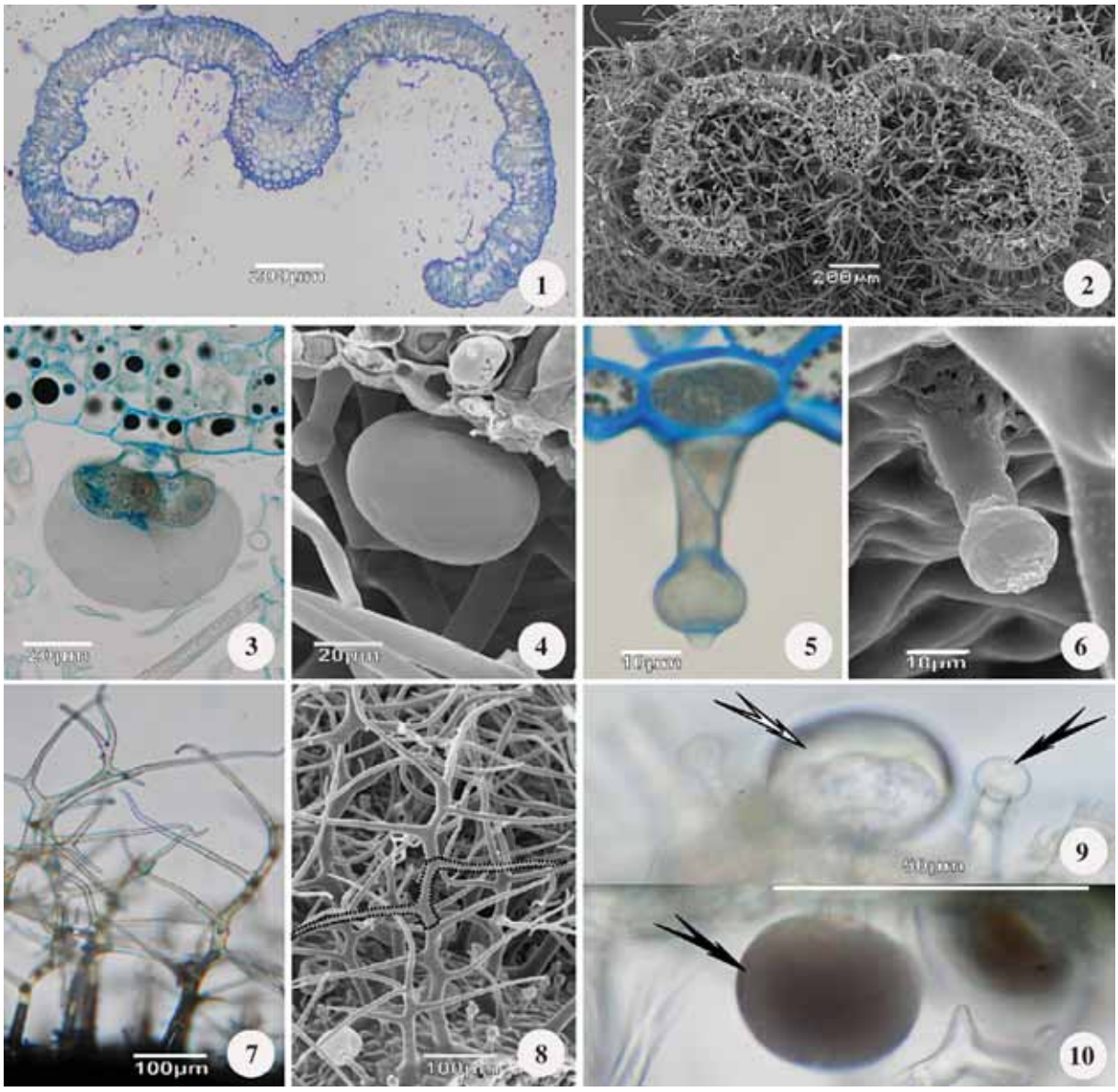
Semi-thin sections of plastic-embedded tissue were stained with: a) 0.5% toluidine blue O in 1% borax solution (O' Brien & McCully, 1981) as a general stain, b) saturated Sudan black B solution in 70% ethanol (Bronner, 1975) for lipids, c) saturated alcian blue solution in 3% acetic acid (Mowry, 1956) for polysaccharides, d) 1% aniline blue black in 70% acetic acid (Fisher, 1968) for proteins.

For fluorescence microscopy (FM), 20 μm-thick sections of fresh samples were cut with a cryotome (Leica CM1850, Germany) at –10°C, embedded in Jung Tissue Freezing Medium (Leica Microsystems Nussloch GmbH, Germany), and examined directly with an Olympus BX40 microscope equipped with a digital camera (DP71, Olympus, Japan). A BP 330-385 exciter filter and a BA 420 barrier filter were used.

Hand sections of fresh leaf tissue were stained with a series of histochemical reagents. Among them: a) osmium tetroxide (Lison, 1960) for unsaturated lipids, b) concentrated H_2SO_4 (Cappelletti *et al*., 1986) for sesquiterpenes, c) vanillin/HCl (Guerin *et al.*, 1971) for flavonoids, d) antimony trichloride ($SbCl_3$) (Hardman & Sofowora, 1972) for terpene- containing steroids, e) Dittmar's reagent (Furr & Mahlberg, 1981) for alkaloids, f) potassium bichromate (Faure, 1914) for tannins, g) alcoholic vanillin-HCl (vanillin test) (Gardner, 1975) for phenolic compounds, h) ferric chloride (Johansen, 1940) for polyphenols, and i) DMB (3,4-dimethoxy-benzaldehyde or veratraldehyde) for phenolic tannin precursors (Mace & Howell, 1974). All stains were matched by controls. All tissues were viewed with an OLYMPUS CX41 light microscope. Micrographs were recorded with a Nikon D300, 12.2 megapixel camera.

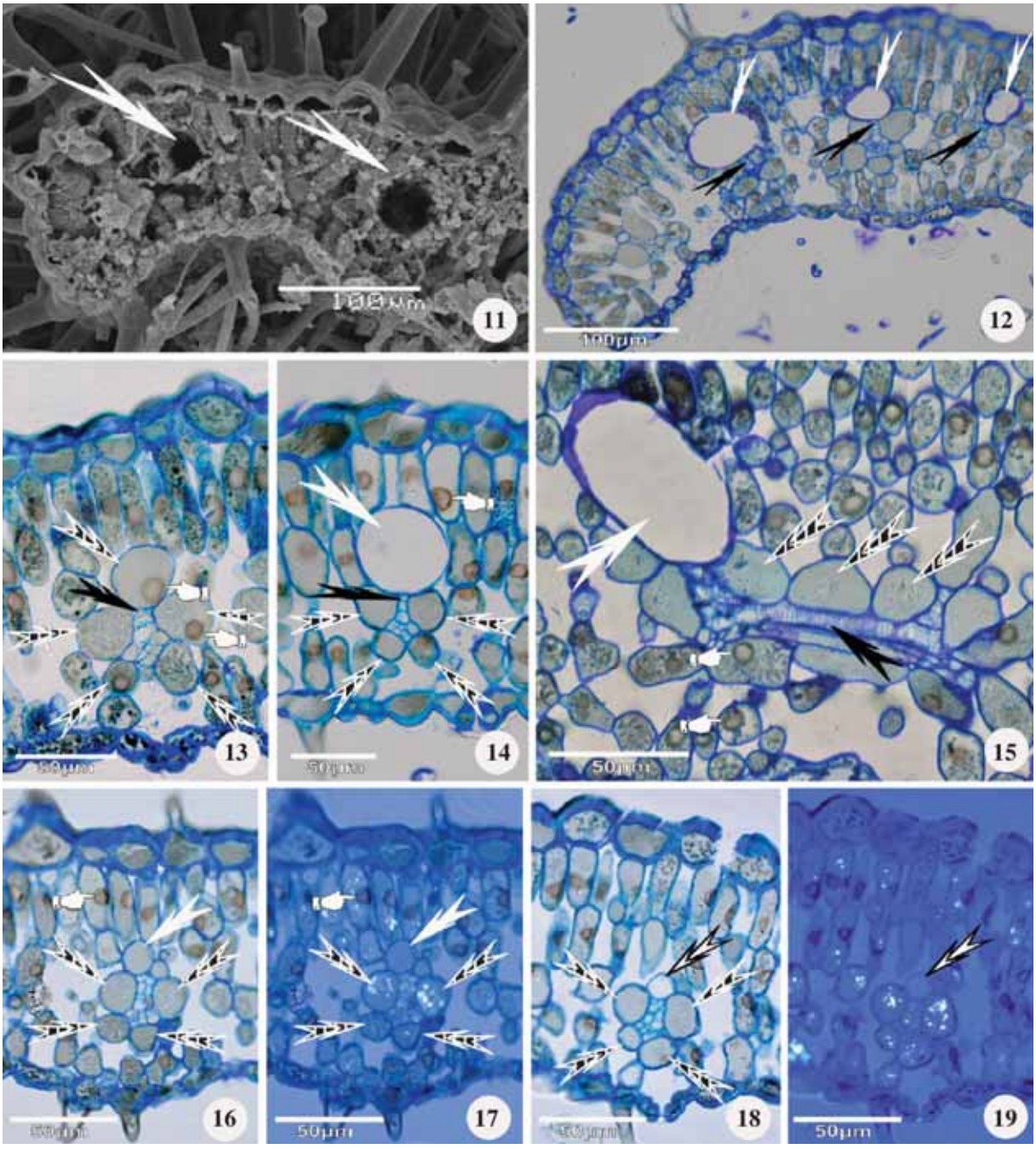
RESULTS

The prominent characteristic of the small, curved leaf of *T. polium* (Figs 1, 2) is, in both light and scanning electron micrographs, the opulent indumentum composed of numerous glandular and non-glandular hairs (Fig. 1). Glandular hairs of the peltate type (Figs 3, 4 and 9, 10) are prominent, yet fewer than those of the capitate type (Figs 5, 6, black arrow in Fig. 9). The former are mostly located on the abaxial site of the



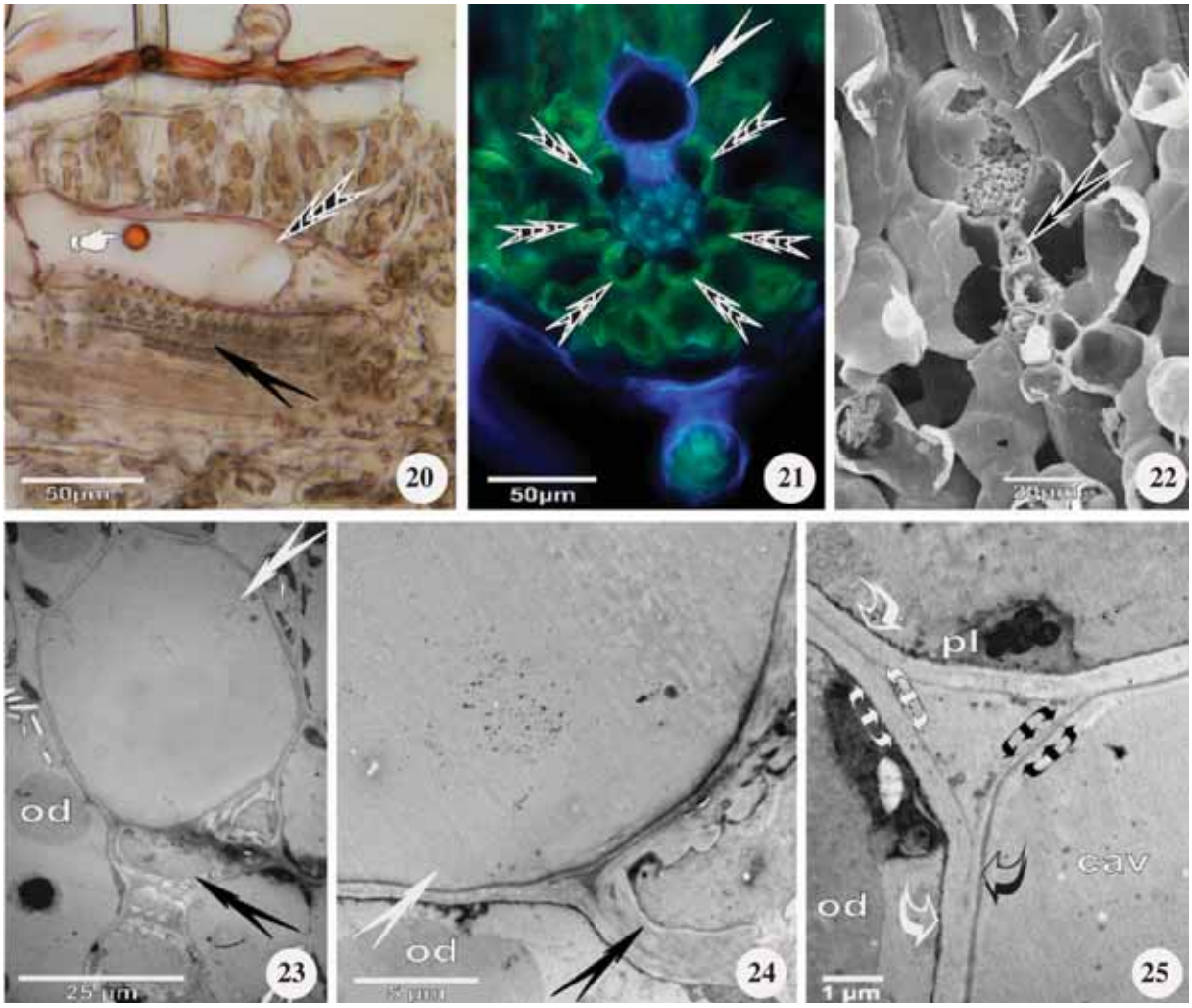
FIGS 1-10. The leaf of *T. polium*.

Fig. 1. Light micrograph of a cross section of *T. polium* leaf.
Fig. 2. Scanning electron (SE) micrograph of a leaf cross section.
Fig. 3. Light micrograph of a peltate hair. The stalk cell, head cells, and the secreted material are evident. Dark globules within the epidermal cells are condensed phenolics.
Fig. 4. SE micrograph of a peltate hair.
Fig. 5. Light micrograph of a capitate hair. The cutinous dome of the head contains the secretion.
Fig. 6. SE micrograph of a capitate hair. The cutinous dome seems deformed.
Fig. 7. Dendroid hairs on the surface of a fresh leaf.
Fig. 8. SE micrograph of the upper leaf surface. One of the cells composing the hair is outlined. A peltate (bottom, left) and a few capitate hairs can be discerned in the picture.
Fig. 9. Fresh leaf section showing a peltate and a capitate hair on the upper leaf surface. Arrows point to the secretory heads. Compare the size of these two hairs.
Fig. 10. Fresh leaf section showing a peltate hair on the lower leaf surface. Notice the opaque material accumulated (arrow).



FIGS 11-19. Cellular cavities in the mesophyll of *T. polium*. White arrows point to cellular cavities, black arrows to the vessel elements of the bundle, striated arrows to the bundle sheath cells. Pointing hands indicate the globular, hydrophobic inclusions of the vacuole.

FIG. 11. SE micrograph of a leaf cross section showing mesophyll cavities.
FIG. 12. Cross section of the leaf close to the margin.
FIG. 13. Cross section of a minor conductive bundle.
FIG. 14. Cross section of a conductive bundle with a developed cavity. Its close association to the uppermost vessel element is evident.
FIG. 15. Paradermal section of the leaf. The cellular cavity is sectioned obliquely. Palisade cells (top, right) appear round.
FIG. 16. Cross section of a minor bundle. The vacuole of the uppermost cell of the sheath is stained like the others.
FIG. 17. Same to FIG. 16, viewed with polarized light. The uppermost cell can be easily marked out for not having crystal inclusions.
FIG. 18. The uppermost cell of the bundle sheath is not stained.
FIG. 19. Same to FIG. 18, viewed with polarized light. No crystal inclusions can be observed within the uppermost cell of the bundle sheath.



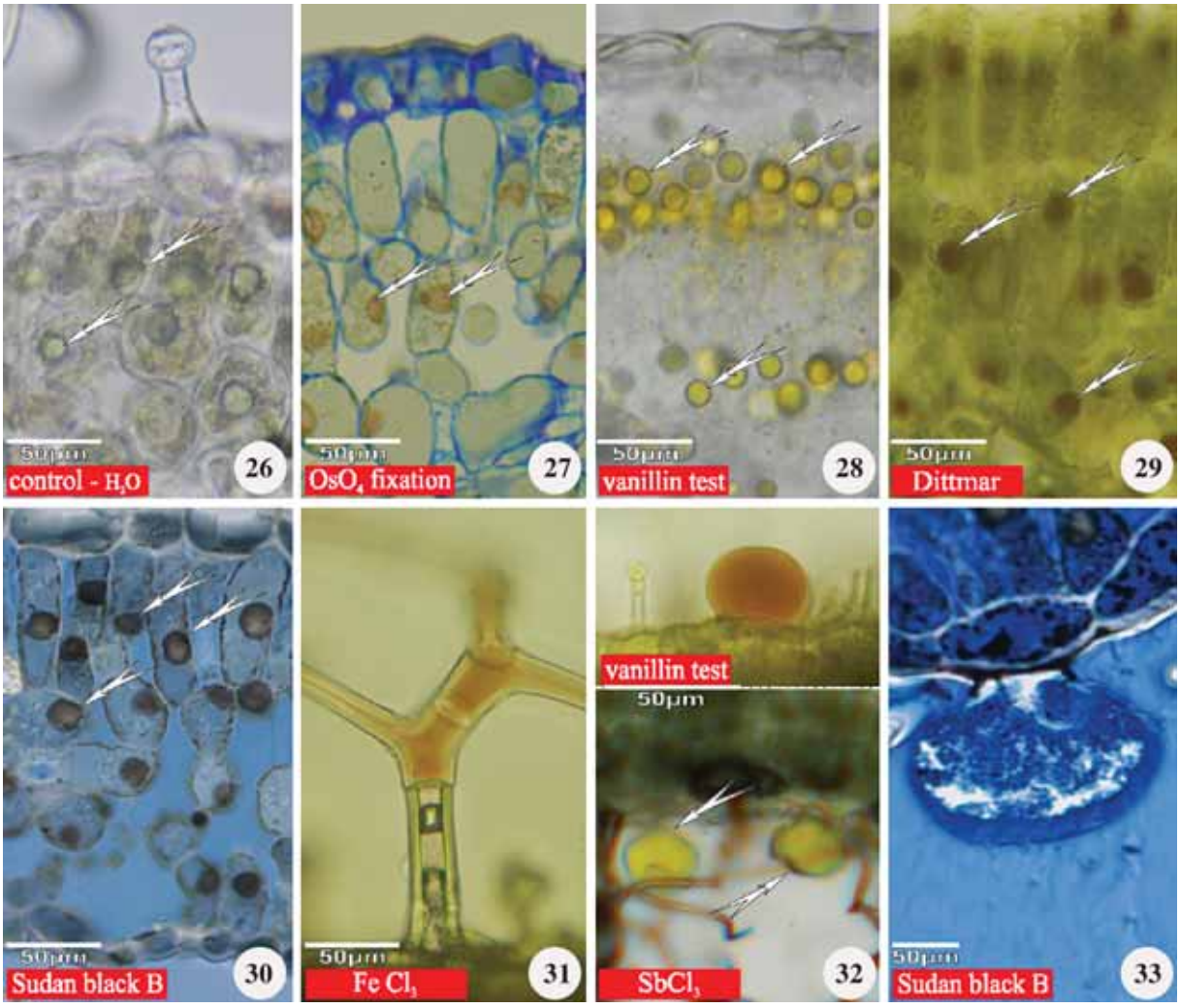
FIGS 20-25. Cellular cavities in the mesophyll of *T. polium*.

Fig. 20. A cross section of a fresh leaf, cut parallel to a conductive bundle. Staining with Sudan III. The stained, cutinous cell wall of the cellular cavity is evident. A lipid droplet within the cell is also stained in dark orange color.
Fig. 21. Fluorescent microscopy. The uppermost cell is the only cell of the bundle sheath to present fluorescence.
Fig. 22. SE micrograph of a section from a leaf fixed after the summer period. The partly broken cell wall allows the observation of the "spongy" vacuolar content.
Fig. 23. Transmission electron micrograph (TEM) of a cell cavity.
Fig. 24. TEM. The cell cavity (white arrow) and the adjacent vessel element (black arrow), appear in close contact.
Fig. 25. TEM. Close-up at the cell walls of the cell cavity and the adjacent bundle sheath cells. od = oil droplet, pl = plastid, cav = cellular cavity. Triple black arrow points to the thin wall of the cell cavity, triple white arrow points to the thick cell wall of the adjacent sheath cell.

leaf (Fig. 10).

Peltate hairs (Figs 3, 4) are composed of a foot cell, a stalk cell, and a multicellular head. The secreted material accumulates between the apical walls of the secreting head-cells and the covering cuticle (Fig. 9). Notably, most of the peltate hairs on the upper leaf surface contain a secretion which appears translucent (Fig. 9) whereas hairs on the lower surface accumulate an opaque, brownish substance (Fig. 10). Intact capitate hairs consist of a unicellular foot, a two-celled stalk and a rounded, unicellular head (Figs 5, 6 and Fig. 11). In these hairs, secretions are stored between the apical wall of the head cell and the raised cuticle. Storage starts as a small dome, at the upper part of the head cell (Fig. 5).

Besides glandular hairs, a large number of long, multicellular, dendroid, non-glandular hairs develop from epidermal trichoblasts, producing a thick pu-



FIGS 26-33. Histochemical treatment of leaves (thick sections of fresh and epoxy-embedded tissue) of *T. polium*.

FIG. 26. Untreated tissue.

FIG. 27. OsO_4 fixation and toluidine staining.

FIG. 28. Alcoholic vanillin-HCl (vanillin test) for phenolic compounds. Although the mesophyll is totally and intensely stained, the thin margins of the sections remain unstained (the contents leach from the cells). In any case, the globules within the palisade cells retain a dark yellow color.

FIG. 29. Dittmar's reagent for alkaloids. Globules are stained dark red.

FIG. 30. Sudan black B staining of plastic-embedded tissue. Globules are lightly stained.

FIG. 31. Positive reaction of the hair cells to $FeCl_3$ for polyphenols.

FIG. 32. Upper: Alcoholic vanillin-HCl (vanillin test). Peltate hairs on the upper leaf surface are stained brown. Lower: $SbCl_3$ staining for terpene-containing steroids. Peltate hairs on the lower leaf surface exhibit a yellow coloration.

FIG. 33. None of the peltate hairs reacts to Sudan black B.

bescence covering the leaf (Figs 7, 8). Each of the cells composing a protective hair has an axial part and one or –most often– two long, spiny projections on either side (dotted line in Fig. 8). These cells are attached onto each other, constructing a single, elongated protective apparatus.

The anatomy of the leaf lamina is rather simple. The adaxial epidermal cells possess a well thickened, densely stained, external periclinal wall (Figs 12 to 14). A single layer of a loose palisade parenchyma (Figs 12, 13, 14) is located below the upper epidermis. The spongy parenchyma occupies approximately half of the mesophyll thickness and most of the mesophyll cells appear to accumulate large droplets (pointing hands in Figs 13, 14 and 15). Numerous granular, osmiophillic inclusions can also be observed in many

mesophyll cells (Figs 13, 15 and 18).

A prominent feature of the leaf is the presence of numerous cavities, ovoid but mainly globular in shape, occurring in the middle of the mesophyll (white arrows in Figs 11, 12, 14 and 15), usually closer to the leaf margins (Figs 11 and 12). Sometimes, they seem to merge producing larger, oval-shaped formations. These cavities are always closely associated to vessel elements of the conductive tissue (black arrows in Figs 12, 14). Each cavity resides at the top of a conductive bundle, attached to the topmost vessel element (Figs 12, 14, 24).

In sections of fresh and plastic-embedded leaf tissue at various orientations, these cavities appear mostly as globular spaces (see paradermal section in Fig. 15). Observing cross sections and series of paradermal sections we may assume that these cavities orientate from the parenchymatous transfer cells of the bundle sheaths. Sheath cells, accompanying the bundles, (Figs 13, 16, 18 cross sections, striated arrows, Fig. 20, longitudinal section, striated arrow) are more or less globular or ovoid (Fig. 15, paradermal section, striated arrows). Their cell wall readily stains with Sudan III (Fig. 20). Sometimes, the uppermost cell of the sheath appears larger (Fig. 13). This cell, in those of the bundle sheaths that have no cavity, is positioned exactly where the globular cavities appear in some others (Figs 13 and 16). It stains identically to the other sheath cells but differs from them since the vacuole deprives of calcium oxalate crystals (HCl soluble, turning to gypsum with H_2SO_4) or other granular inclusions (Figs 13, 16 and 17) abundant in the neighboring sheath cells. Sometimes, the uppermost sheath cell appears to have no content (Fig. 18) –as the cell cavities do appear– and still differs from the other sheath cells for not having any inclusions (Fig. 19). In any case, the cell cavities are attached to the upper vessel element and in contact to their neighboring cells of the bundle sheath (striated arrows, in all pictures). They are of cellular origin (not intercellular spaces), their cell wall is cutinized as it becomes clear with fluorescence microscopy (Fig. 21) while in the late summer leaves they appear impregnated with a material of spongy texture (Fig. 22). The nature of this material could not be defined. It is probably soluble water since all attempts for histochemical localization –in sections of fresh and epoxy-embedded tissue– failed to give any reaction.

In transmission electron micrographs, the cell cavities appear empty (Fig. 23). They are in close contact to the vessel members (Fig. 24). Their cell wall, significantly thinner (black, triple arrow in Fig. 25) than the wall of the adjacent sheath cells (white, triple arrow in Fig. 25), is lined with an electron dense layer from within (black, bended arrow in Fig. 25) which probably has to do with the impregnation of the wall with cutin while from the side of the adjacent sheath cell, a layer of highly osmiophillic phenolics seems to line the wall (white, bended arrows in Fig. 25).

The results of the histochemical investigations can be summarized in the localization of phenolics (vanillin test) within the vacuoles of leaf cells, terpene-containing steroids ($SbCl_3$) in most of the hairs on the lower leaf surface (Fig. 32, lower) and terpenes (H_2SO_4/C_2H_5OH) within the head cell of the capitate hairs. A faint positive reaction for the contents of the hairs on the upper leaf surface indicated accumulation of phenolic compounds (Fig. 32, upper). Lipids were not traced in any of the secretory hairs (Sudan black B, negative, reactions Fig. 33). Vanillin-HCl gave a vivid reaction for flavonoids in the mesophyll. The droplets accumulated within most of the mesophyll cells (pointing hand in Figs 13, 14, 15 and 16, white arrows in Figs 26 to 30), they were tinted brownish in OsO_4 fixed tissues (Fig. 27), deep yellow with vanillin test (white arrows in Fig. 28) and dark red with Dittmar's reagent (white arrows in Fig. 29) in fresh tissue while they stained light grey against the blue background in sections of plastic-embedded tissue treated with Sudan black B (white arrows in Fig. 30). Phenolic tannin precursors (DMB stain), polysaccharides, and proteins did not seem to accumulate in any of the leaf cells. Epidermal cells showed a faint positive reaction to $K_2Cr_2O_7$, for tannins. The numerous dendroid hairs, on both surfaces, accumulate tannins within the thick walls of their cells (Fig. 31).

DISCUSSION

The "light" structure of the leaf of *T. polium*, with only a single layer of palisade cells, seems contrasting to an expected abundance of palisade parenchyma so far believed as necessary for increasing the CO_2 absorbing surface of the mesophyll in xerophytes (Rhizopoulou & Psaras, 2003; Terashima *et al*., 2005). Both summer and winter leaves of the seasonally dimorphic sub-shrubs (i.e. *Cistus* sp., *Euphorbia acanthothamnos*, *Phlomis fruticosa*, *Thymus capitatus*, *Origanum vulgare*, etc.) present a multilayered, well developed palisade parenchyma (Christodoulakis & Bazos, 1990). Moreover, the rather loose spongy parenchyma and the absence of prominent, well developed

mechanical tissue, do not suggest commitment to the xeromorphic structure. Stomata were not observed on the upper surface, even though amphistomaty is considered a major xeromorphic character (Fahn & Cutler, 1992). This character is thought to reduce the distance of CO_2 diffusion to mesophyll cells (Terashima *et al*., 2005). However, amphistomaty is totally excluded from the mediterranean evergreen sclerophylls and appears only sporadically on the winter leaves of some seasonally dimorphics (*Anthyllis hermaniae*, *Thymus capitatus*). Therefore, *T. polium*, although growing in the phryganic formations, seems to follow the evergreen sclerophyll pattern.

Considering the above-mentioned leaf features, we may suggest that *T. polium* diverges, in some way, from the typical xeromorphic characters and probably compensates for this "deficiency" by taking advantage of the protection given by the heavy pubescence. However, thick and complex hairs are common among the plants of the mediterranean region, especially among the seasonally dimorphics (Christodoulakis, 1989; Christodoulakis & Fasseas, 1991). The thick pubescence may also play a protective role against ultraviolet B radiation as is the case of the leaves of *Olea europaea* and *Quercus ilex* (Karabourniotis *et al*., 1992, 1993; Skaltsa *et al*., 1994; Karabourniotis & Fasseas, 1996).

Another major response of the mediterranean plants against stressful environmental conditions is the production of secondary metabolites which, sometimes, are very useful to humans (Nikolakaki & Christodoulakis, 2004, 2007). The presence of these metabolites is an indication of the plant's defensive reaction –eventually its adaptation– to the stressful environmental conditions. Such metabolites are produced by the secretory hairs as well. It is interesting that the secretory activity seems to vary among the peltate hairs of this plant. Most of the hairs located on the lower leaf surface produce an opaque, brownish material. By contrast, hairs on the upper leaf surface produce an essential oil that seems to remain trapped in the space between the apical cell wall and the cuticle of the secreting head cells (compare Fig. 9 to Fig. 10). Antunes *et al.* (2004) do not mention these hairs at all. Capitate hairs are also active in secretion. Considering the obvious activity of the numerous peltate and capitate hairs and pointing out that they react positively to different histochemical treatments, we may assume that the leaf pubescence produces a range of different metabolites.

Besides secretory hairs, the large cavities within the mesophyll seem to be of interest. Each of them is in contact to a vessel element of a conductive bundle. They possess a typical, cutinized, but thin cell wall which indicates that they originate from an expanded single cell. The cavities of *T. polium*, superficially, seem very similar to the secretory structures (oil cells) observed within the leaf of *Laurus nobilis* (laurel) (Fahn, 1988; Christodoulakis & Fasseas, 1990), although the oil cells of laurel are by no means in contact to the conductive tissue and never merge. Observations of fresh tissue failed to trace the nature of the contents. No staining was possible within these cavities with any of the histochemical reagents, even in thick sections where these cavities could remain intact. Their content could either be depleted in fresh sections or appear inactive in histochemically investigated sections of plastic-embedded tissue because it was removed, probably as water soluble, during fixation. It cannot be of hydrophobic nature, since some signs of it would be observed in the form of tiny droplets in fresh sections or in epoxy-embedded tissue, even if this content was readily dissolved in acetone during tissue dehydration. Of great interest is the fact that leaves fixed at late summer (September) appear, in scanning electron micrographs, to have these cavities filled with a material of spongy texture. Yet, even in these leaves, no histochemical reaction turned positive for the content of these cavities.

Transmission electron microscope observations offer additional evidence supporting the cellular origin of these cavities. They are large, mature cells with cutinized cell wall. The presence of cutin is the reason for the intense fluorescence of this wall yet, despite cutinization, the materials accumulated probably leach from the vacuole during tissue treatment. The contact of the cavities to the vessel elements of the conductive tissue probably indicates the reliance of these cells to the water flow.

Lersten and Curtis (1998) reported the presence of similar "idioblasts" in two species of the family Lamiaceae. The idioblasts they observed can be traced everywhere in the mesophyll. Sometimes they appear isolated. They are not necessarily in contact to the conductive bundles and if they are, this can happen at any position of the bundle. On the contrary, the cavities of *T. polium* appear in contact to the bundles, always at the upper part where the uppermost vessel element is located. Moreover, they are always positioned as bundle sheath cells. Lersten and Curtis (1998) also reported that the "idioblasts" they observed ap-

pear to be empty and therefore, they consider them as dead cells. In *T. polium*, at the end of summer, cellular cavities seem to contain a non-homogenous ("spongy") material indicating that they are living cells exerting osmotic pressure to the surrounding parenchyma cells.

The nature and the location of the cellular cavities of *T. polium*, their obligatory affinity to the xylem, the structure of their wall, and the results of the histochemical assays favor the suggestion of a "water reservoir" function. If this is true then a unique, great advantage for the leaves of *T. polium* in resisting the mediterranean arid summer is obvious. Yet further investigation would be interesting since, besides the possibility that these cells store water, they may also hinder the passage of water in some way, as it was suggested by Fahn & Cutler (1992).

Histochemical reagents assisted in revealing that terpenes, alkaloids, and tannins are mainly produced within the leaf cells and the secretory hairs. Flavonoids were rarely traced. Although the reagents used present medium specificity, if matched by controls they are considered as standard for such investigations and no references for false reactions or artifacts exist.

The numerous hydrophobic droplets, accumulated within most of the mesophyll cells, appear interesting in some way. Their reaction to various histochemical stains indicates that they could be a mixture of lipids, phenolics, and alkaloids. All these compounds are considered as hydrophobic (Borgnia *et al*., 1996). All other small, dark, granular, extremely osmiophillic contents of the vacuoles (Figs 13 and 15, top right and Fig. 18, top) of the leaf cells are the condensed tannins (high molecular weight, polymerized phenolics), commonly found in mediterranean plants and other xerophytes. These metabolites are very hulking and are dispersed within the whole vacuole.

Concluding, we could say that the production of terpenes, flavonoids, and phenolic compounds seems to be –once more– a part of the leaf's defensive response against the arid and hot environment of the mediterranean region (Fahn, 1988; Fahn & Cutler, 1992; Christodoulakis, 1989, 1992; Christodoulakis & Fasseas, 1991). This provides a good reason for the wide use of this plant for pharmaceutical purposes. On the other hand, *in vitro* cultured cells are generally able to synthesize and accumulate most of the secondary metabolites of high commercial and pharmaceutical value detected in parent plants (Dronne *et al.*, 1999; Nikolakaki & Christodoulakis, 2004). Given that and the fact that tissue cultures can be considered as potential secreting factories (Ramachandra Rao & Ravishankar, 2002), the present investigation may show the way, through the localization of the sites where useful metabolites are synthesized, to the *in vitro* cultures of secretory cells for the production of novel compounds from low-cost precursors (Ramachandra Rao & Ravishankar, 2002; Nikolakaki & Christodoulakis, 2007).

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