The capitate and peltate glandular trichomes of *Lavandula pinnata* L. (Lamiaceae): histochemistry, ultrastructure, and secretion[1]

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Abstract:

The morphology, histochemistry, and ultrastructural aspects of the secretion of *Lavandula pinnata* L. (Lamiaceae) glandular trichomes were studied in order to prepare for work on calcium distribution in normal and heat stressed plants. The secretory process was observed in both light and dark-grown plants. The secretion of the capitate glandular trichomes consists of both lipophilic and polysaccharidic substances (mainly the latter), while peltate glandular trichomes only secrete lipophilic substances. Our ultrastructural results are consistent with the finding that plastids are the main organelle that synthesizes the lipophilic substances, especially terpenes, while dictyosomes synthesize polysaccharides. The essential oil is most likely transported directly by the RER, while the polysaccharides reach the plasma membrane via Golgi-derived vesicles. Exocytosis is the main way that secretory products move from the cell to the subcuticular space. There are no differences in gland development or secretion between the light and dark-grown plants.

Article:

Lavender is an important ornamental and essential oil producing plant used in the perfume, cosmetic, flavoring, and pharmaceutical industries. Most *Lavandula* species only grow in Mediterranean climates, prefer warm, sunny, dry weather, and dislike hot, humid conditions. Compared to other species of *Lavandula*, *L. pinnata* L., which grows wild in the arid regions of Canary Islands, can tolerate hot weather and can be planted in the lower latitudes of countries such as China and Japan.

Different species of Lamiaceae have different types, distribution, morphology, and density of glandular trichomes, which can be important taxonomic characters (Schnepf 1968, El-Gazzar and Watson 1970, Heinrich et al. 1983). The glandular trichomes are termed peltate or capitate, depending on the structure of the secretory head (Werker 1993). In general, capitate glandular trichomes have one or two secretory, disk cells, while peltate trichomes may have up to eight cells in the disk. In both cases, the secretory substance is accumulated in a subcuticular space, outside of the cell wall. Peltate trichomes produce most of the essential oil, with terpenes

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comprising the main component (Clark et al. 1997, Turner et al. 2000). Along with small amounts of other monoterpenes and sesquiterpenes, the essential oil β-Phellandrene is a main component of the secretory product (Figueiredo et al. 1995). In addition to small amounts of essential oil, capitate trichomes normally secrete varying amounts of polysaccharides (Werker 1993).

A good deal of research has been done on trichome structure in relation to the secretory process, in order to elucidate the secretory mechanism (Amelunxen 1965, Bosabalidis and Tsekos 1982, Bourett et al. 1994, Figueiredo and Pais 1994, Ascensa˜o et al. 1997, Gersbach 2002). Most of this research has focused on only a few species, such as *Origanum dictamnus* L (Bosabalidis and Tsekos 1982), *Perilla ocymoides* (Danilova and Kashina 1987), *Leonotis leonurus* (Ascensa˜o et al. 1995), *Salvia officinalis* (Corsil and Bottega 1999), *Mentha X piperita* (peppermint, Turner et al. 2000), and *Prostanthera ovalifolia* (Gersbach 2002). This research has shown that most organelles participate in the production or storage of the essential oil. This includes the plastids (Amelunxen 1965, Wooding and Northcote 1965, Akers et al. 1978), endoplasmic reticulum (ER) (Schnepf 1972, Bourett et al. 1994, Ascensa˜o et al. 1997, Turner et al. 2000), and vacuoles (Amelunxen 1965). Exocytosis is the most probable mechanism for movement of the essential oil from the cell to the subcuticular space (Ascensa˜o and Pais 1998, Gersbach 2002).

More recently, attention has turned to the regulation of glandular trichome development, and the regulatory metabolism of the essential oil. Both developmental and environmental factors are known to influence the yield and composition of essential oil, with consequences for the commercial production of this commodity (Burbott and Loomis 1967, Clark and Menary 1979, Dudai et al. 1992, Sudria et al. 1999, 2001).

Although there has been little work on the glandular trichomes of *Lavandula* spp., Huang et al. (2005) studied the morphology and developmental anatomy of the trichomes of *L. pinnata* and found that, unlike other Lamiaceae species, both types of trichomes have spherical heads (Figs. 1A–1C, 1K). We have retained the term "peltate" for the trichomes with the larger heads in order to avoid confusion with the literature.

In this paper we present the structure and development of the secretory glands of *L. pinnata* in preparation for work on calcium distribution in normal and heat stressed plants. Because part of our study of calcium distribution was conducted with a calcium channel blocker (nifedipine) that is inactivated by light, we have also examined gland development and ultrastructure in dark-grown plants. We hope this study will add to the knowledge of the morphology, histochemistry and the ultrastructural aspects of the secretion of glandular trichomes.

MATERIALS AND METHODS

PLANT MATERIALS.

Axillary buds from the middle of the stem and measuring 2 to 3 mm in length were excised in the prefloral state from one-year old *Lavandula pinnata* L. plants cultivated in a greenhouse at the South China Botanical Garden (voucher 697119, IBSC). The seeds from which these plants were grown were purchased from Kew Gardens, UK. The explants were thoroughly washed in 70% ethanol for 1 min, and subsequently surface-sterilized with l g l21 sodium hypochlorite for 15 min, followed by five rinses in sterile distilled water. The sterile explants were implanted

vertically on Murashige and Skoog (MS) culture medium in 2 cm diameter glass tubes. Murashige and Skoog medium consists of MS salts (Murashige and Skoog 1962), 3% sucrose and 0.6% agar. For the initial multiplication, the MS medium was supplemented with 0.5 mg l21 (2 µM) 6-Benzylaminopurine (Tsuro et al. 2001). The cultures were split and half were maintained at 25 ± 2°C with 16-hour day length at a light intensity of 10–20 mmol $_m$22 s^{-1} (Phillips TLD 36W/84), while half were maintained in the dark. After about 20 days' growth, during October 2005, samples of these materials were selected for study (see below). Light-grown material was used for all methods, while dark- grown samples were only observed with Transmission Electron Microscopy (TEM).

LIGHT MICROSCOPY

Fresh leaves at various stages of maturity (1–5 mm) were selected for observation from light-grown cultures. The distribution and external morphology of the secretory trichomes was examined and photographed with a Zeiss Stemi SV II stereo microscope fitted with a Zeiss AxioCam MRC digital camera.

HISTOCHEMISTRY AND FLUORESCENCE MICROSCOPY

Fresh, cultured leaves (ca. 1 cm) were cut into approximately 1 mm^2 using razor blades and used for the following histochemical tests: Sudan III for total lipids (Jensen 1962), Periodic Acid-Schiff (PAS) reagent for polysaccharides (Jensen 1962), and Ruthenium Red for pectin (Johansen 1940). Controls consisted of unstained cells. Squares were also mounted in water on glass slides with cover slips, and examined by fluorescence microscopy for lipids, especially terpenoids, at wavelengths of 334 and 365 nm. Observations were made on a Zeiss Axioplan 2 microscope using both transmitted light and epifluorescence.

TRANSMISSION ELECTRON MICROSCOPY

(TEM). Cultured leaves at different developmental stages (1–5 mm) from both light and dark-grown plants were cut into approximately 1 mm^2 and fixed in 2% glutaraldehyde in 0.1 M phosphate buffer (pH 7.0) in separate glass vials. Low vacuum was used at room temperature for 1 h until the tissue was infiltrated with fixative, and fixation was continued for a further 12 h at 4 °C, without vacuum. After fixation, the tissue was washed three times with 0.1 M phosphate buffer, 15 min per wash. The squares were post-fixed in 1% osmium tetroxide for 2 h, followed by a further three 15 min washes in buffer, and a final wash for 15 min in distilled water. Dehydration was in a water/ethanol series (15, 30, 40, 50, 60, 70, 80, and 90%) for 1 h each, followed by three changes of anhydrous ethanol for 1 h each. The dehydrated sections were infiltrated with 10, 20, 40, 60 and 80% Spurr's resin in anhydrous ethanol for 6 h each, followed by five changes of pure resin for 6 h each. The squares were then embedded in flat moulds using fresh resin, and polymerized at 70 °C for 24 h. Ultra-thin sections (80 nm) were cut using a Leica-Ultracut S ultramicrotome, and stained with uranyl acetate. Three trichomes per leaf were randomly selected for analysis and photography with a JEM-1010 TEM, operating at 100 KV (Gersbach 2002). All materials were processed for observation by the first author in the shared Electron Microscopy Facility of the South China Botanical Garden.

RESULTS

The distribution of glandular trichomes of *Lavandula pinnata* is apparently random, with about 16 capitate trichomes mm^{-2} , and 6.5 peltate trichomes mm^{-2} on the abaxial side of fully expanded leaves (Figs. 1A and 1B). Both capitate and peltate trichomes are composed of a basal

cell (BC), a stalk cell (SC) and apical disk secretory cells (DC) (Figs. 1C, 1K). There are one (Fig. 1G) or two (Fig. 1C) secretory cells in a capitate glandular trichome, but eight secretory cells in a peltate glandular trichome (Fig. 1L). A mature capitate trichome at the secretory stage is only about in 25 µm in height and 20 µm in diameter. A mature peltate trichome is four times this size, with a height of ca. 120 gm, a basal cell height of 50–70 gm, and a diameter of ca. 70 gm.

HISTOCHEMISTRY.

The secretory products of capitate glandular trichomes stain positively for both lipophilic (Figs.1C, 1G) and hydrophilic substances (Figs. 1D, 1E, 1H, 1I). The stalk cells respond positively to lipophilic compounds (Figs. 1C, 1G), but negatively to hydrophilic compounds (Figs. 1D, 1H, 1I). The peltate trichomes' secretion responds positively to stains for lipophilic compounds (Fig.1K, L), and negatively to stains for hydrophilic compounds (Fig. 1M).

The secretory (disk) cells of the capitate trichomes show green autofluorescence under UV excitation (Fig. 1F), a normal autofluorescence color for epidermal cells. In the peltate trichomes, the cell walls of both the basal and disk cells emit a yellow-green autofluorescence (Figs. 1J, 1N), which results from the cutinization of the walls. At the secretory stage, the disk cells of these trichomes contain lipids, which appear as bright dots under UV excitation (Fig. 1J, arrows). At the post-secretory stage, the secretory substances in the subcuticular space emit blue light under UV (Fig. 1N), which indicates the presence of terpenoid compounds.

ULTRASTRUCTURE AT THE EARLY PRE-SECRETORY STAGE.

Three different stages of secretion can be recognized: pre-secretory, secretory, and post-secretory. The pre-secretory stage includes all developmental phases prior to the inception of gland filling. During this stage both capitate and peltate glandular trichomes are initiated from a single protodermal cell. This cell becomes the apical cell of the trichome, and divides one to three times to produce the two (capitate) to eight (peltate) head cells. The onset of the secretory phase is marked by the beginning of secretion and a separation of the thickened cuticle from the cell walls across the apical surface of the gland. Post-secretory glands with filled subcuticular

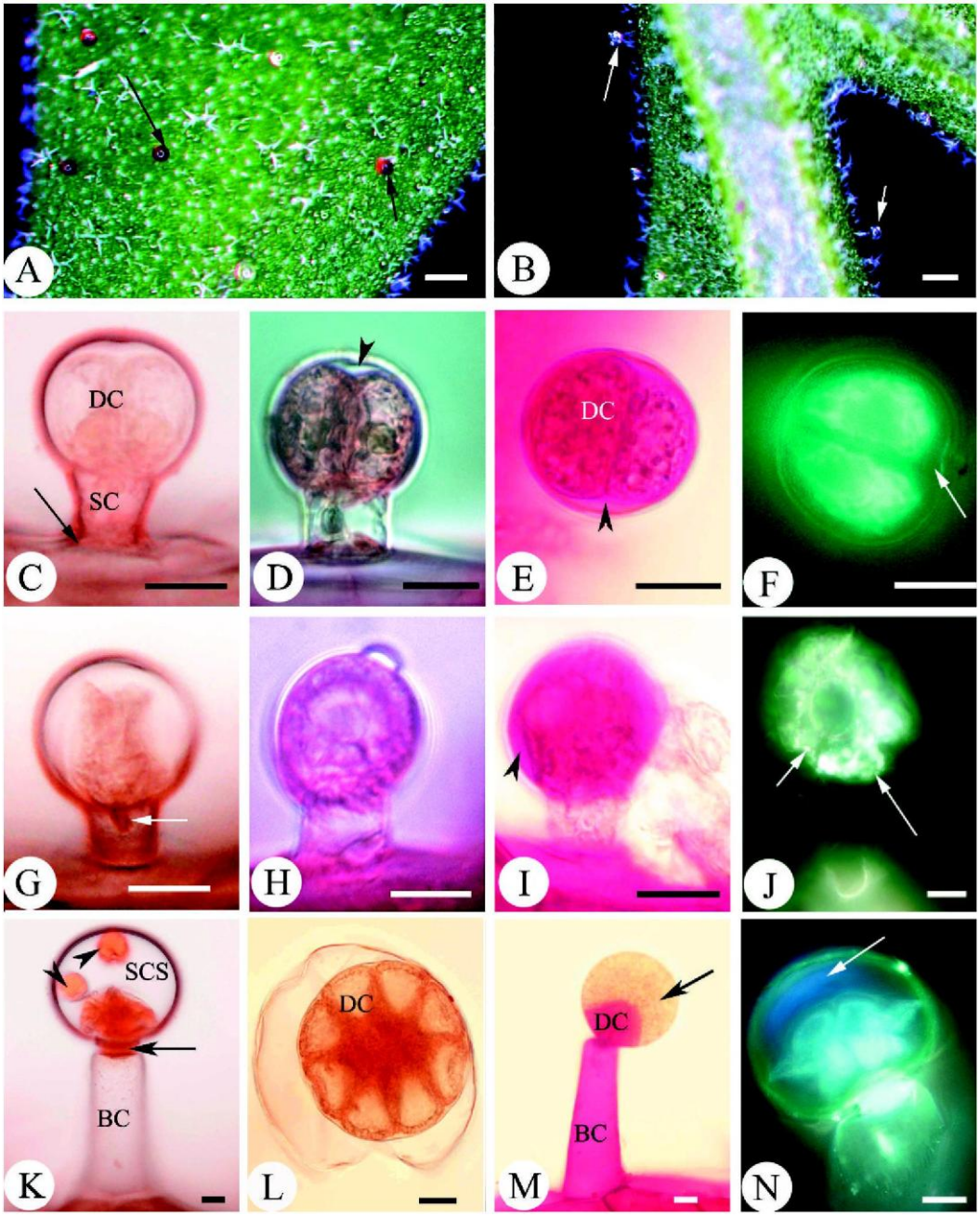

FIG. 1. Light micrographs of capitate and peltate glandular trichomes. A: Abaxial view of mature leaf tip showing the characteristic orange to brownish color, size, and distribution of peltate trichomes (arrows). B: Lateral view of peltate trichomes showing their spherical heads (arrows). C–E, G–I, K–M: Histochemical characterization of the glandular trichomes. D, H, L: Fluorescence microscopy images. C–F: Capitate trichomes with two-celled heads. E and F are polar, other views are lateral. C: Disk (DC) and stalk cells (SC) stained for total lipids with Sudan III. The basal cell (arrow) is unstained. D: Disk cells stained for pectin with Ruthenium Red. The stalk cell and the secretion in the subcuticular space (arrowhead) are negative to Ruthenium Red. E: Disk cells and secretion in subcuticular space (arrowhead) stained for polysaccharides with PAS. F: Disk cells emitting a bright green autofluorescence under UV excitation, indicating the presence of lipids. The subcuticular space (arrow) is black indicating the absence of lipids. G–I: Capitate trichomes with one-celled heads. All views are lateral. G: Stalk cell stained for lipids (arrow) with Sudan III. The secretion in the subcuticular space is Sudan III-negative, indicating the absence of lipids. H: Disk cell stained for pectin with Ruthenium Red. The presence of pectin is indicated by red staining. I:

Disk cell and secretion in subcuticular space stained for polysaccharides with PAS. The stalk cell is PAS negative. J–N: Peltate trichomes. J, K, M, N are lateral views, L is polar. J: Disk cells of a peltate trichome during the secretion stage, emitting yellow-green autofluorescence under UV excitation. The bright spots are lipids (arrows). K: Post-secretory stage lipids stained with Sudan III (arrowheads). The disk (DC) and stalk cells (arrow) are Sudan III-positive, while the basal cell (13C) remains unstained. L: Lipids (orange) in the eight head cells stained with Sudan III at the post-secretory stage. M: The walls of disk, stalk and basal cells are positively stained for polysaccharides with PAS, but the secretion in the subcuticular space (arrow) is negative. N: Post- secretory stage. The secretory product in the subcuticular space (arrow) emits a blue autofluorescence under UV excitation indicating that it contains terpenoid compounds. The lateral walls and cuticle emit a yellow autofluorescence, indicating the presence of the cutin layer. Scales = 150 μm (A, 13); 10 μm (C–N).

spaces appear as spherical domes (Figs. 1K, 1M) inserted in slight depressions in the leaf epidermis.

At the beginning of their development, and through the three-celled stage, it is difficult to distinguish capitate and peltate glandular trichomes (Figs. 2A–C). At their initiation, both arise as protruding epidermal cells, which appear meristematic. These cells have few small vacuoles, relatively large nuclei, large nucleoli, numerous ribosomes, and many mitochondria and proplastids (Fig. 2A). The cytoplasm becomes asymmetrically distributed as the cells enlarge so that the cells have vacuolated basal portions, and cytoplasmically dense apical portions (Fig. 2A). The tips of the cells contain many vesicles, mitochondria, and short and sparsely distributed rough endoplasmic reticulum (RER). The proplastids contain only small amounts of lipid (Fig. 2D). At the two-celled stage, the apical cells maintain their meristematic-like character, while the basal cells vacuolate (Fig. 213). The nucleoli are still very large at this stage. At the three-celled stage, the apical cells retain their meristematic-like appearance, but the stalk and basal cells are vacuolated (Fig. 2C). During these stages, the apical cells show scarce Golgi stacks, with most vesicles close to the plasma membrane (Fig. 2E). This arrangement of vesicles is consistent with the finding that the Golgi plays a role in plasma membrane synthesis. After the early pre-secretory stage, many differences appear between the two kinds of glandular trichomes.

ULTRASTRUCTURE OF THE SECRETORY PROCESS IN LIGHT-GROWN CAPITATE TRICHOMES

The ultrastructure of the secretory processes is similar in both one- and two-celled capitate trichomes. Here we describe the process in trichomes with two secretory cells.

At the pre-secretory stage, after the apical cell divides to produce a two-celled apical disk (Fig. 3A), the plastids become larger and the Golgi stacks increase in size. Many short cisternae of rough endoplasmic reticulum (RER), which are usually restricted to the cell periphery, appear in the interior of the cell (Figs. 3A, 313). Small, granulose (Fig. 3A, arrows) and osmiophilic deposits (lipids) (Fig. 3A, arrowheads) appear in the proplastids. Plasmodesmata occur frequently between the two disk cells, and also occur between the basal cell and the adjacent leaf cells, but are less numerous. The outer cell walls of the head develop a layer of cutin (Fig. 313, black arrowheads). Golgi stacks, consisting of seven to nine flattened cisternae (Fig. 3C) are commonly assembled in groups in cytoplasmic regions rich in both RER and polyribosomes (Figs. 313, 3C). Toward the end of the pre- secretory phase, the basal and the stalk cells develop central vacuoles and the cuticle begins to thicken, particularly on the lateral walls of the stalk. At the secretory stage, osmiophilic substances appear in the cytoplasm, and a layer of secretory product occupies the space between the cell wall and cuticle (Figs. 3D, arrowheads). At this

stage, large lipid droplets appear in the stroma of plastids of the disk cells (Fig. 4A), the RER occur in greater profusion, and the Golgi bodies are more abundant (Fig. 413, arrowheads). Lipid spherosomes also appear (Figs. 413, 4C). Deeply stained RER cisternae are found appressed to, and secretory vesicles are tightly associated with, the plasma membrane, suggesting exocytosis. A very sinuous plasma membrane with deep invaginations is also observed (Fig. 4D). Osmiophilic substances are observed in membrane-bound vesicles, the external membranes of which are fused

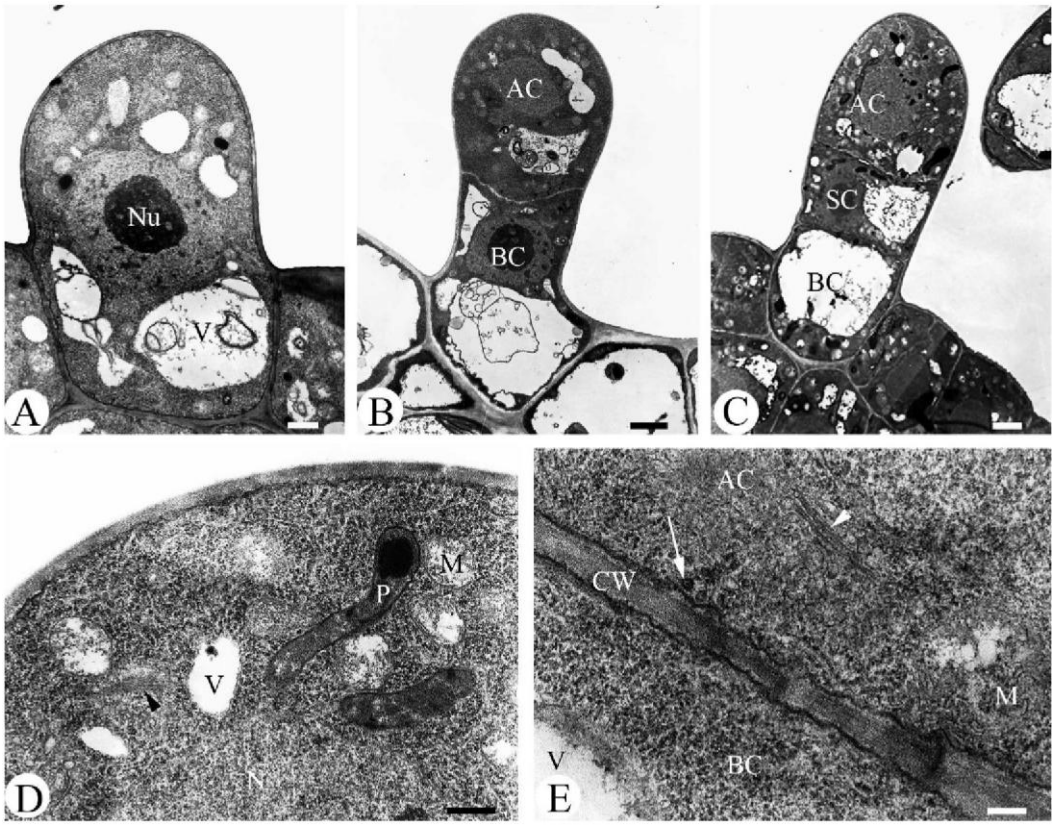

FIG. 2. TEM micrographs of glandular trichomes at the early pre-secretory stage. A: The initial cell of a glandular trichome contains relatively large nuclei and nucleoli (Nu), numerous ribosomes, and proplastids, but is not highly vacuolated (V). B: Young trichome after one periclinal division, with a vacuolate basal cell (BC) and a densely cytoplasmic apical cell (AC) with few vacuoles. C: Three-celled stage of a glandular trichome. The apical cell (AC) retains its meristematic-like character, but the stalk (SC) and the basal cells, (BC) are vacuolated. D: The apical portion of an initial cell containing mitochondria (M), Golgi bodies (arrow head), vacuoles (V), and proplastids (P). RER is relatively sparse. N = nucleus. E: Portions of the apical (AC) and basal cell (BC) at the two-celled stage, showing scarce Golgi stacks (arrowhead), mitochondria (M), and vesicles close to the plasma membrane (arrow). CW = cell wall, V = vacuole. Scale = 2 μm (A–C), 500 nm (D), 200 nm (E).

with the plasmalemma releasing the substances into the periplasmic space (Figs. 4D, 4E). On this evidence, exocytosis is the probable mechanism for secretion. As secretion continues, the cuticle begins to separate from the cell wall to form a subcuticular space, which gradually fills with secretory product (Fig. 4E, white arrow). At this stage, RER are still abundant, as are osmiophilic substances in the plastids (Fig. 4F).

During the post-secretory stage, the subcuticular space is full of lipophilic (Fig. 4G) and polysaccharidic substances (Figs. 1I). We did not find any naturally broken capitate trichomes.

ULTRASTRUCTURE OF THE SECRETORY PROCESS IN LIGHT-GROWN PELTATE TRICHOMES.

During the pre-secretory stage, the heads enlarge from two to eight cells. At the two-celled and four- celled stages, the proplastids are small, amoeboid and contain small osmiophilic droplets; the RER is relatively sparse, and Golgi bodies are rare. Although the cuticle has formed, it remains thin (Figs. 5A, 5B). As the head forms eight cells, the plastids enlarge and RER begin to increase in frequency in the parietal cytoplasm and become the dominant component of the secretory cells. During this stage, the RER consist of long narrow cisternae, forming stacks parallel to each other, and to

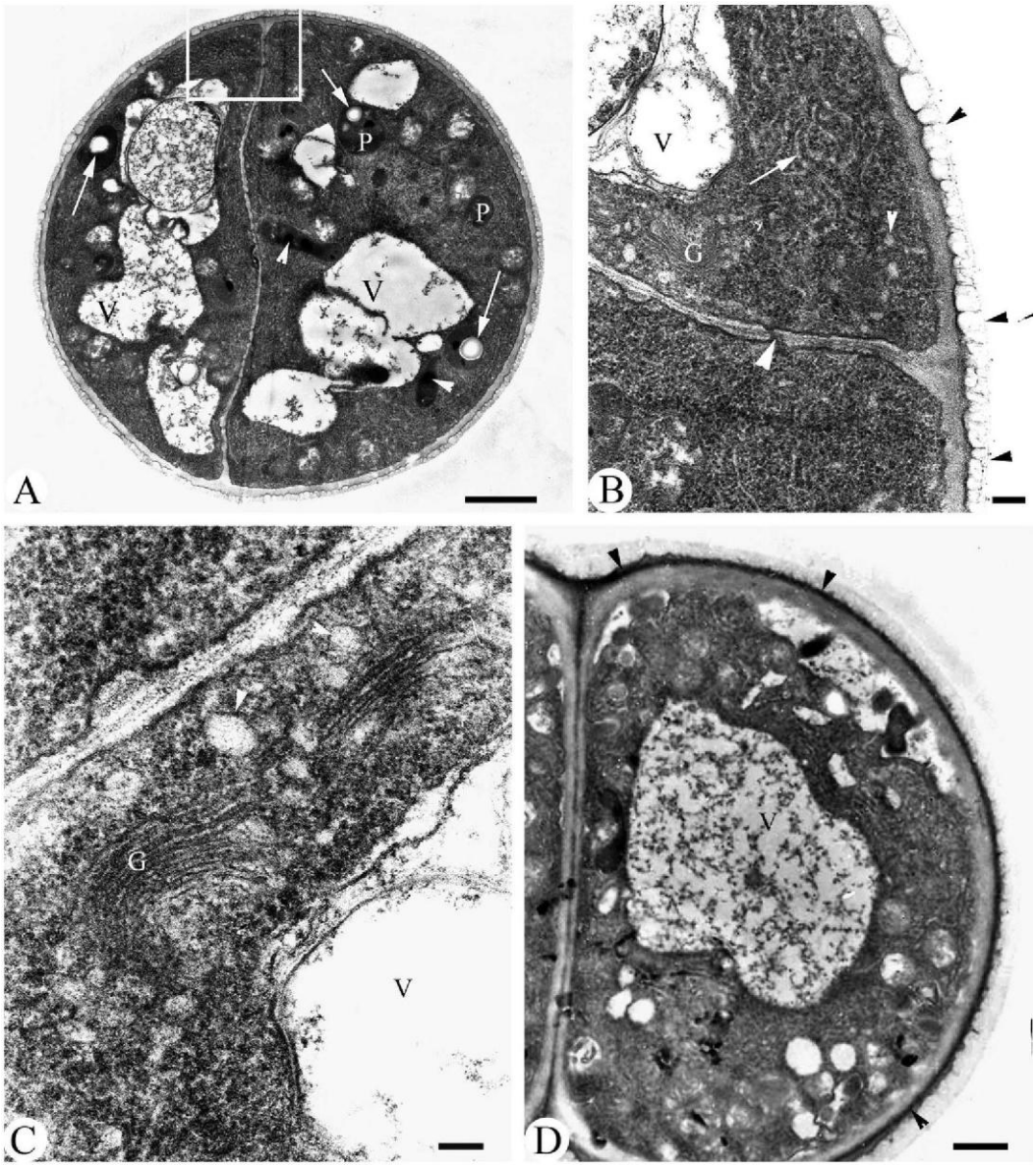

FIG. 3. TEM micrographs of periclinal sections of capitate trichomes. A: Trichome with two disk cells, at the pre-secretory stage. Vacuoles (V) are more abundant and larger than at earlier stages, and the plastids (P) are larger and contain embedded granules (arrows) and small osmiophilic deposits (arrowheads). B: Enlarged portion of Fig. 2A (box) showing abundant rough ER (arrow), Golgi bodies (G), Golgi vesicles (small white arrowhead) and vacuoles

(V). Plasmodesmata (large white arrowhead) appear between the disk cells, and a cutin layer has formed (black arrowheads). C: The pre-secretory stage, showing an increased number of Golgi stacks (G), and vesicles (arrowheads) close to the plasma membrane. V = vacuole. D: Trichome at the secretory stage. A layer of secretory product (arrowheads) has formed between the cell wall and cuticle. V = vacuole. Scale =2 µm (A), 200 nm (B), 100 nm (C) 1 gm (D).

the plasma membrane (Fig. 5C). The plastids are restricted to the peripheral cytoplasm, and the cuticle thickens (Fig. 5C).

At the secretory stage, the disk cells enlarge and are characterized by numerous vacuoles of various sizes, enlarged plastids, many extended RER, and a few Golgi bodies (Figs. 5D, 5E). There is also a subcuticular space, full of secretory product. Osmiophilic materials appear in the cytoplasm, and in the small

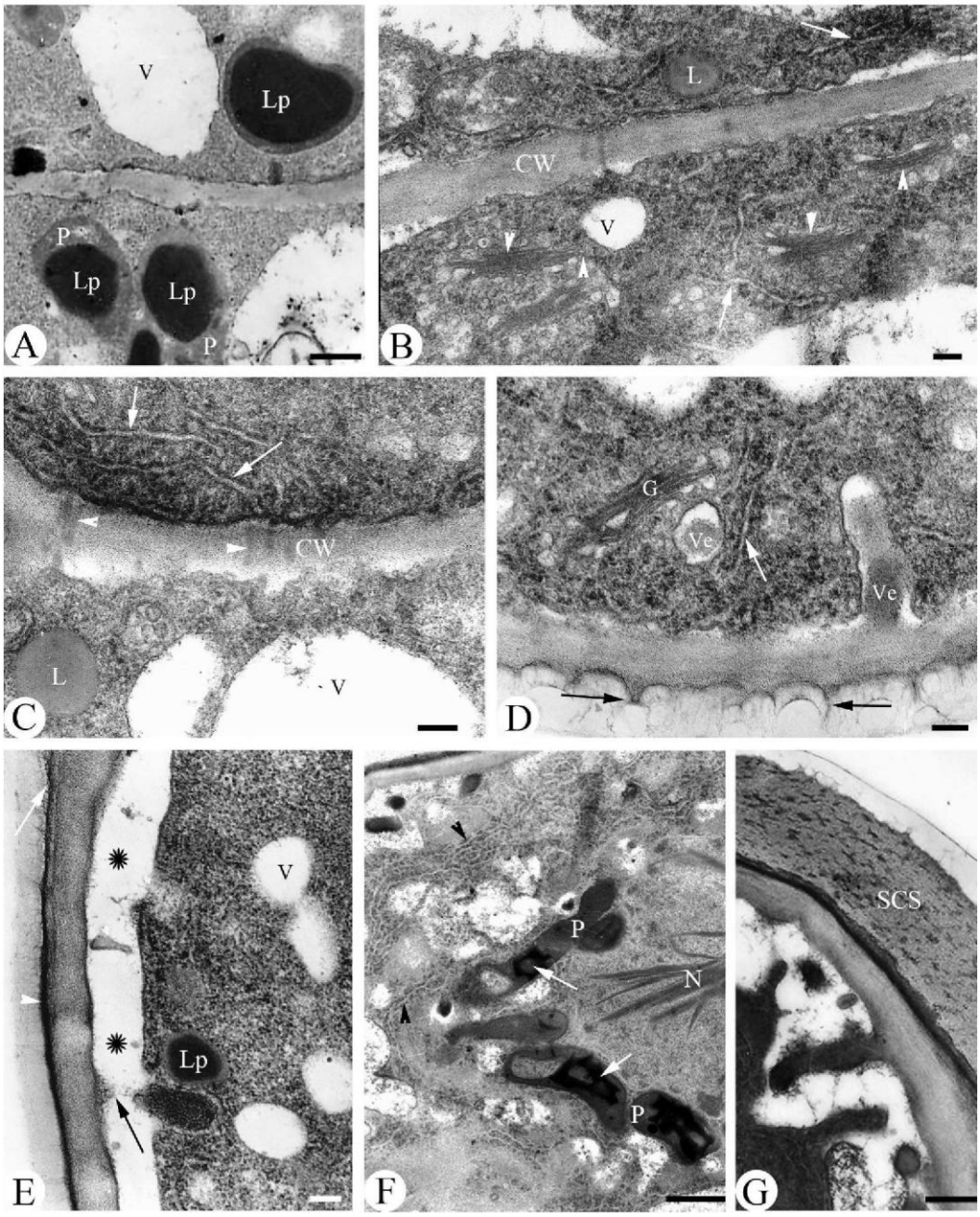

FIG. 4. TEM micrographs of head cells of capitate trichomes during the secretory and post-secretory stages. A: Secretory stage with lipid droplets (Lp) present in the plastids (P). M = mitochondria. B: Secretory stage showing abundant Golgi bodies (arrowheads) and RER (arrows) near the cell walls (CW). Lipid spherosomes (L) also occur in the disk cells. V = vacuole. C: Secretory stage with abundant RER (arrows) appressed to the plasma membrane, and fibrillar-like osmiophilic substance (arrowheads) in the cell wall (CW). L = lipid spherosomes, V = vacuole. D–F: Secretory stage. D: Golgi stacks (G) in close association with RER cisternae (arrows). Large Golgi-vesicles (Ve) with osmiophilic substance are close to, and fusing with the plasma membrane. Pectocellulosic fibrils are evident (arrows) in the cuticle. E: The lipid droplets (Lp) entering into the periplasmic space (stars) and attaching to the cell wall (black arrow). As the secretory product increases (dark area at arrowhead), the cuticle begins to separate from the cell wall to form a subcuticular space (light space at white arrow). F: Plastids (P) containing osmiophilic material (arrows). RER (arrowheads) are still very abundant. N = nucleus. G: Post-secretory stage, showing the subcuticular space (SCS) full of secretion. Scale = 500 nm (A), 200 nm (B–E, G), 1 µm (F).

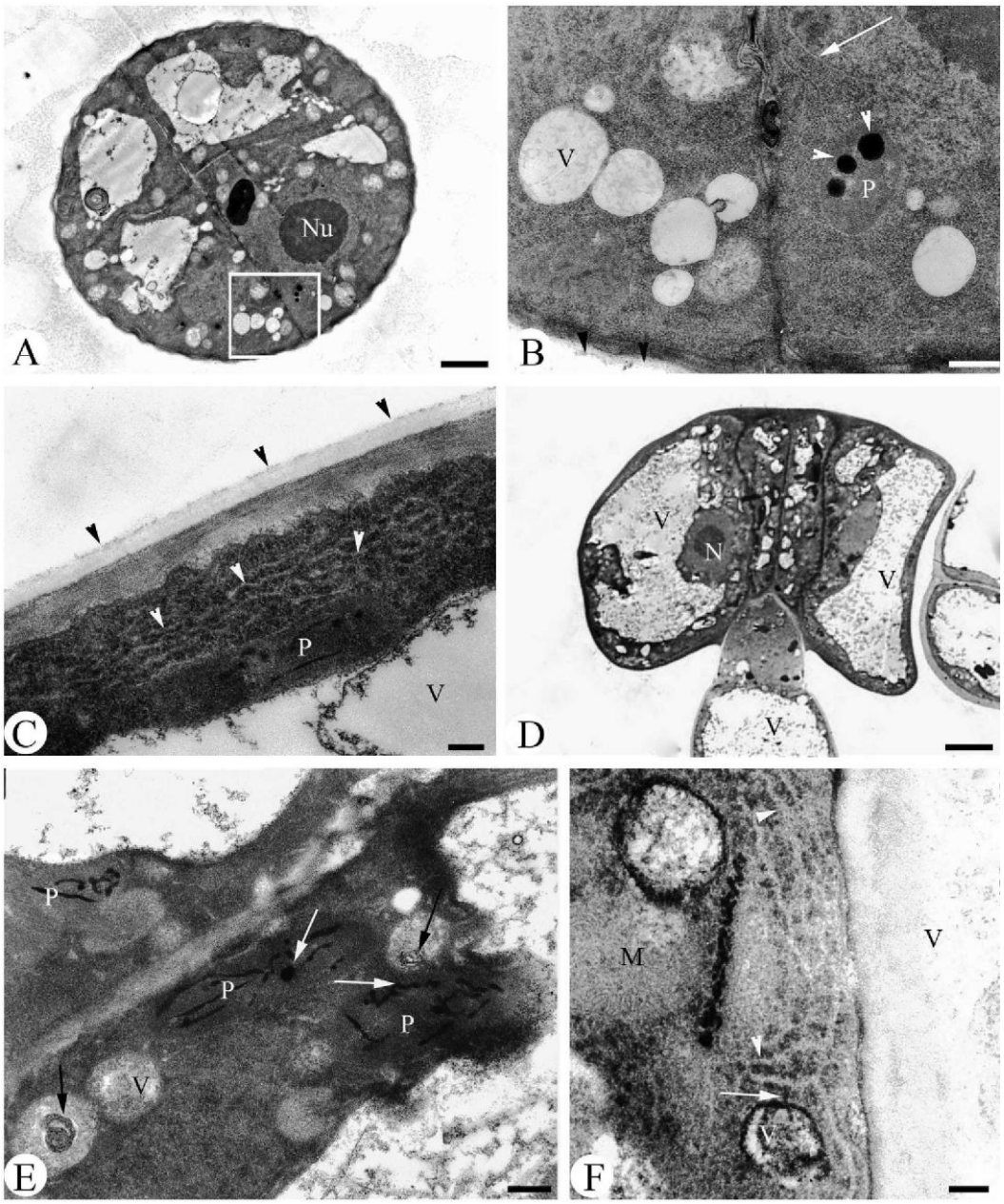

FIG. 5. TEM micrographs of peltate trichomes. A: Trichome head with four-cells, at the pre-secretory stage. The cells have relatively large nuclei and nucleoli (Nu), and appear meristematic. B: Enlarged portion of Fig. 5A (box), showing relatively sparse RER (arrow), small proplastids (P) some of which contain osmiophilic deposits (arrowheads), and a thin layer of cuticle (black arrowheads). V = vacuole. C: Peripheral region of a peltate glandular trichome at the pre-secretory stage. The well-developed RER (white arrowheads) are arranged in strands parallel to the plasma membrane, and are near plastids (P). The cuticle is thicker than at previous stages (black arrows). V = vacuole. D: Secretory stage showing large disk cells characterized by numerous large and small vacuoles (V). N = nucleus. E: Secretory stage showing osmiophilic material (white arrows) in vacuoles, and being transferred from plastids to vacuoles (black arrow). P = plastid. F: Secretory stage showing osmiophilic material (arrows) being transferred to vacuoles (V) after processing by RER (arrowheads). M = mitochondria. Scale = 2 µm (A), 500 nm (B, E), 200 nm (C, F), 7 gm, (D).

vacuoles located near the plastids and RER (Figs. 5E, 5F). The presence of osmiophilic materials in these vacuoles is validated by their autofluorescence with UV excitation (Fig. 1J). The osmiophilic materials appear to be transferred directly from the plastids (Fig. 5E, black arrow) and RER (Fig. 5F, arrow). Unlike the capitate glandular trichomes, no large vesicles were observed fusing with the plasmalemma at any developmental stage.

ULTRASTRUCTURE OF THE SECRETORY PROCESS IN DARK-GROWN GLANDULAR TRICHOMES.

Growth in the dark did not influence gland initiation or development in either type of trichome. The gland initial cell shows no significant differences from the control, and neither the ultrastructure of the head cells, the structure of plastids, RER, mitochondria nor the distribution of the Golgi bodies are affected by dark treatment. As in the light- grown plants, the plastids contain lipid droplets, large vesicles occur in the capitate trichomes, the RER are abundant in the peltate trichomes, and the subcuticular spaces of both trichomes are full of secretory product.

DISCUSSION

The presence of both capitate and peltate glandular trichomes is one of the features that distinguishes the Lamiaceae from other families. Capitate trichomes generally have rounded to pear-shaped heads of one or two cells, supported by stalks of variable length (Ascensão et al. 1999). Peltate trichomes have short one-celled stalks and large flattened heads of ca. 60–90 µm in diameter, which are formed by four or eight cells arranged in a simple disk (Amelunxen and Gronau 1969, Antunes and Sevinate-Pinto 1991, Serrato-Valenti et al. 1997), or by 12– 18 cells in two concentric circles (Werker et al. 1985a, b, Hanlidou et al. 1991).

Within the family, different species can have both peltate and capitate trichomes, only peltate, only capitate, or more rarely, neither. Zheng (2001) reports only one type of glandular trichome on the leaves of Amenthystea caerulea, their heads consisting of secretory cells in two layers. In addition to peltate trichomes, Corsil and Bottega (1999) identified four different types of capitate trichomes on the leaves of *Salvia officinalis*. Each type has a different spatial arrangement of cells, a different function, and secretes a different combination of lipophilic and hydrophilic substances. In contrast, only long and short- stalked capitate trichomes are present in *Siderites syriaca* (Karousou et al. 1992), and *Leonotis leonurus* (Asensão et al. 1995). Both of these trichomes have four-celled heads, and narrow subcuticular spaces.

The peltate trichomes of *Lavandula pinnata* differ from those reported in other species in that the basal cells are relatively long, ca. 50– 70 µm, nearly one and one half times the length of cells in other species. In terms of the head shape, the peltate trichomes of *L. pinnata* have spherical heads, while in other species the head is more truly peltate (Amelunxen and Gronau 1969, Antunes and Sevinate-Pinto 1991, Werker 1993, Turner et al. 2000, Gersbach 2002). These differences suggest that glandular trichome structure could be an important taxonomic characteristic. However, more work on the structure and classification of the trichomes must be done before this potential can be realized.

The capitate and peltate trichomes of *Lavandula pinnata* differ in morphology, ultrastructure, and the composition of their secretory products. At the secretion stage, the capitate trichomes are characterized by abundant RER, Golgi bodies, and large vesicles attached to the cell membrane,

while the peltate trichomes lack Golgi bodies and large vesicles. This suggests that there is higher exocytosis activity in the capitate than the peltate trichomes.

Histochemical studies and UV light excitation indicate that the secretion of the peltate trichomes is an oleoresin that contains terpenoids. Similar secretory products are reported in other plants (Dev et al. 1982, Gersbach 2002, Valkama et al. 2003). The exudate of the capitate trichomes contains polysaccharides and a few lipophilic compounds. This result is consistent with the phytochemical data available for other species of the Lamiaceae (Werker et al. 1985a, b, Dudai et al. 1988, Ascensão and Pais 1998, Ascensão et al. 1999). We also found that the stalk cells of both trichomes contained lipophilic compounds.

Our results are consistent with the finding that Golgi bodies have an important role in polysaccharides biosynthesis in secretory trichomes (Ascensão and Pais 1998, Turner and Croteau 2004). The fact that Golgi vesicles contain polysaccharides was first established in other types of cells (Northcote and Pickett- Heaps 1966, Schnepf 1972, Jones and Morre 1973, Dupree and Sherrier 1998, Dhugga 2005), and confirmed for capitate glandular trichomes in studies of other species (Amelunxen 1965, Schnepf 1972, Danilova and Kashina 1987). Large vesicles present near the plasmalemma in *Lavandula pinnata*, in close proximity with the Golgi, suggests that the polysaccharide component of the secretory product is transported to the cell surface via Golgi vesicles, and released into the periplasmic space by exocytosis. A similar mechanism of granulocrine secretion has been reported in other trichomes (Unzelmann and Healey 1974, Kristen and Lockhausen 1985, Meyberg 1988, Vassilyev 1994a, b, Ascensão and Pais 1998, Turner and Croteau 2004).

Early studies of Lamiaceae glandular trichomes reported the synthesis of lipophilic substances in the vacuoles (Amelunxen 1965), plastids (Amelunxen 1965, Wooding and Northcote 1965, Akers et al. 1978), and SER (Schnepf 1972). More recent studies reported the synthesis of these substances in plastids, and their transport to the SER (Bourett et al. 1994, Ascensão et al. 1997, Turner et al. 2000, Turner and Croteau 2004, Machado et al. 2006). Although we did not observe the presence of SER, abundant RER is found at the secretory and post-secretory stages. The RER may have a role in the biosynthesis, accumulation and secretion of terpenes (Gunning and Steer 1975, Skubatz et al. 1995, Valkama et al. 2003).

Osmiophilic deposits occur in the vacuoles of the peltate trichomes of *Lavandula pinnata*, based on UV light excitation and TEM. Early work took the presence of these types of deposits as support for the hypothesis that vacuoles have a part in essential oil synthesis (Amelunxen 1965). In *L. pinnata*, osmiophilic substances are transferred from the plastids and RER to the vacuoles. Therefore, we presume that vacuoles can not produce, but only process these substances (Zheng et al. 2002, Machado et al. 2006).

Our observations support the hypothesis that there are two routes of lipophilic secretion (Zheng et al. 2002). In the first, lipophilic substances produced by plastids are processed and transported by ER vesicles, and released into the subcuticular space by exocytosis (Turner et al. 2000, Zheng et al. 2002). In the second, lipophilic substances are enclosed in small vacuoles, where they are further processed. The small vacuoles fuse to produce larger vacuoles, or fuse directly with the

plasmalemma and release the lipophilic substances into the subcuticular space (Rechmilevitz and Fahn 1975, Ding et al. 1996).

Our study shows that the peltate trichomes are the main structures that secret the essential oil in *Lavandula pinnata*. This finding is in agreement with previous work, which shows that the peltate trichomes produce most of the essential oils in the Lamiaceae (Amelunxen 1965, Werker et al. 1985b, Werker 1993, Bourett et al. 1994, Clark et al. 1997), although the capitate trichomes also produce a few lipophilic compounds (Ascensão and Pais 1998, Ascensão et al. 1999, Corsil and Bottega 1999).

Additional studies are needed to determine whether the stalk cells can secrete essential oil, and to clarify the physiological relationship between the disk and stalk cells. In most glandular trichomes the stalk cells contain lipophilic substances (Ascensão and Pais 1998, Ascensão et al. 1999, Corsil and Bottega 1999), but the thick cuticle may prevent these substances from being secreted as part of the essential oil (Fahn 1988).

The present study shows that there is little effect on gland development or secretion from relatively short dark treatments. The morphology and ultrastructure of the glands, along with the shape and structure of the organelles such as plastids, mitochondria, and RER, are the same as in the normally light-grown seedlings.

CONCLUSION

This paper represents the first detailed comparative analysis of leaf glandular trichome morphology and ultrastructure in *Lavandula pinnata*. The morphology of the peltate trichomes of is different from other Lamiaceae in that the heads are spherical. However, the development and secretory ultrastructure of both capitate and peltate trichomes is similar to that of other species that have been studied. Both types of trichomes undergo several cell divisions, thickening of the cuticle, and an enlargement of the subcuticular space as the oil begins to accumulate. The plastids, RER, Golgi, and vacuoles all participate in the synthesis of secretory products, which are released into the subcuticular space by exocytosis. We did not discover any influence on the secretory process from growth in the dark.

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