**CHAPTER 7**

# TRICHOME MORPHOLOGY AND ULTRASTRUCTURE OF *HELICHRYSUM CAESPITITIUM*

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### 7.1 Introduction

Trichomes are appendages of diverse form, structure and function (Upfold, 1962). Despite the variety of systems that exist for the classification of trichome types, they are ultimately classified as being either glandular with a secretory function or covering hairs (non-glandular) without a secretory function (Cutter, 1978). Developmental and structural studies of trichomes can shed light on the nature of the secreted material and the functional significance of the glands (Franceshi and Giaquinta, 1983). The development of trichomes from the epidermis results from differential enlargement and subsequent divisions of epidermal cells and their derivatives (Carlquis, 1958). In his classification of different trichome types, Upfold (1962) used the plane of division of the initial epidermal cell as a distinctive characteristic. Where more than one trichome type occurs in a single species, each apparently has a special development pathway, as the different structural forms are not one type which is arrested at different stages in a common pathway (Hammond and Mahlberg, 1973). The morphology and ultrastructure of these trichome features have not been reported in *Helichrysum caespititium* (DC.) Harv.

The source of epicuticular secondary metabolites has been attributed to glandular trichomes (Wollenweber, 1984). Production of epicuticular phloroglucinols with antimicrobial properties have been eported in other species of *Helichrysum* (Tomas-Barberan *et al.,* 1988; 1990; Tomas- Lorente *et al.,* 1989; Dekker *et al.,* 1983; Mathekga *et al.,* 2000). Some of these species are in common use in African traditional medicine for the treatment of various ailments. For example, *H. aureonitens* is used against herpes simplex virus type 1 (Meyer *et al*., 1996); *H. melanacme* against drug resistant TB (Lall and Meyer, 1998). Secretions of *H. caespititium* are believed to be effective against

broncho-pneumonial diseases, sexually transmitted diseases, tuberculosis, ulceration and is used as a styptic wound dressing (Phillips, 1917; Watt and Breyer-Brandwijk, 1962).

*H. caespititium* is usually sold on local markets in a fragmentary state. This situation renders this crude drugs highly susceptible to adulteration and substitution. Thus, wrong plant material could easily be used in many herbal preparations with potentially dangerous consequences. The problem of accurate identification and dearth of reliable information about the medicinal plant species found in a region whose flora is incompletely known, have hampered the optimal utilization of these crude drugs and diminished their general acceptability.

In this investigation we examined the morphology and ultrastructure of foliar appendages of *H. caespititium* with a transmission (TEM) and scanning (SEM) electron microscopy. The aim is to evaluate the epicuticular morphology and ultrastructural features of the leaf for reliable taxonomic characters that may facilitate an accurate and rapid identification of the plant sample, and to relate our observation to their, possible functional role in the production of antimicrobial compounds.

## 7.2 Materials and Methods

### 7.2.1 Plant material

Plants used in this study were collected in the Mont-Aux-Sources area in QwaQwa, South Africa during August 1998. A voucher specimen (AM11) of the species was deposited in the herbarium of the National Botanical Institute of South Africa in Pretoria. *H. caespititium* is a prostate, perennial, mat-forming herb that is profusely branched and densely tufted (Figure 1.1). Branchlets are about 10mm tall and closely leafy. Leaves are patent, on average 5-10 x 0.5mm, linear and obtuse with a broad base and clasping branches. Margins are revolute with both surfaces and stems enveloped in a silver 'tissue-paper-like' indumentum, breaking down to wool. The leaves are dotted with orange glands.

### 7.2.2 Transmission electron microscopy

Fresh leaves were sectioned into tip, middle and base portions and immediately fixed in a 2.5% glutaraldehyde: 2% formaldehyde (50:50) mixture in 0.075 M phosphate buffer (pH 7.4), on the collection site. Leaf sections were rinsed three times in the laboratory in 0.075 M buffer and post-fixed in 1% aqueous osmium tetroxide for four hours. The leaf material was then rinsed three times for 10 minutes per rinse in distilled water, and then dehydrated in an ethanol series (50% x 3, 70% x 3, 90% x 3 and 100% x3), for 15 minutes per rinse.

### 7.2.3 Scanning electron microscopy

Cross and longitudinal sections of leaves dehydrated in a graded ethanol series were dried to a critical point with a Bio-Rad E3000 critical point drying apparatus for 24 hours to allow for the substitution of ethanol with $CO_2$. The dried material was mounted, secured by a double-sided adhesive tape (Figure 2A), and carefully examined before suitably representative parts were selected for photography. Each sample was photographed at a magnification of x 750 to reveal the general surface micromorphology and x2500 to show details. Samples were examined with a JEOL 840 SEM at 5kV

## 7. 3 Results

Abaxial and adaxial surfaces as well as cross and longitudinal sections of *H. caespititium* were investigated . Representative scanning electron and transmission micrographs of leaf sections are shown in Figures 7.1 to 7.3.

Observations with the SEM revealed the presence of two types of trichomes. The non-secreting type are abundant and responsible for the silvery 'tissue-paper-like' indumentum covering aerial shoot surfaces. Secreting hairs are club-shaped orange glandular structure of variable size and density found scattered on both surfaces of the leaf. The cuticle is designed to keep water and solutes in, but to keep invaders out. Light microcopy revealed

that the glandular trichomes contain typical secretory cell organelles, including, numerous endoplasmic reticulum, golgi bodies, scattered mitochondria, plastids, ribosomes, and a dense cytoplasm.

The indumentum of long non-glandular trichomes forms a dense covering that completely obscures the epidermal surface and characters (Figure 7.1A-E and G) of the lamina. The non-glandular hairs consists of uniseriate cells displaying a single morphological form (Figure 7. 1A-E and G-H)whereas mature non-glandular trichomes consist of four cells, namely, a base and two stalk living cells and a long dead head cell (Figure 7.1E, 7.2H ). A secreting trichome consists of four cells, namely, a base and two stalk cells and a dome shaped secreting head cell. The base and two stalk cells are characterized by dense cytoplasm, numerous organelles and scattered vacuoles, whereas the dome-shaped apical cell is in addition visibly highly vacuolated 7.3E-G. Patterns of variation in abaxial and adaxial epidermal characters of *H. caespititium* cannot be studied exclusively by LM observations for finer details without the aid of clearing and magnifying devices, because of the dense, protective indumentum

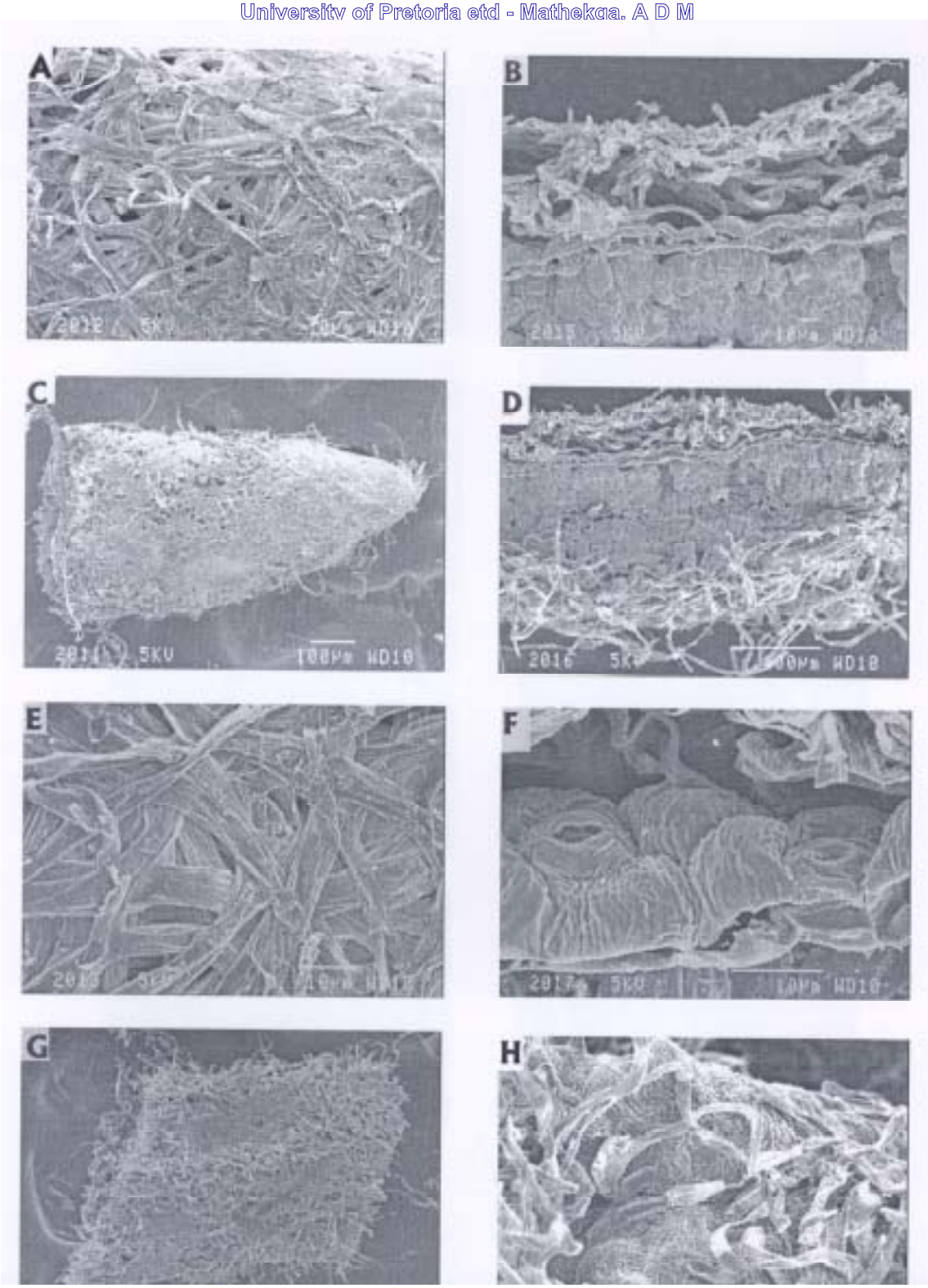


*Figure 7.1 Electron micrographs of leaf epidermal cells of H. caespititium. A. Adaxial indumentum of non-glandular trichomes. B. Cross-section of leaf revealing palisade and mesophyl tissues. C. Leaf tip in indumentum. D. Longitudinal section revealing adaxial and abaxial mesophyls. E. Abaxial indumentum. F. Striated cuticle. (Note that the cuticle is devoid of wax covering and the elevated stomata). G. Middle section of leaf in indumentum. H. Partially cleared abaxial epidermis.*

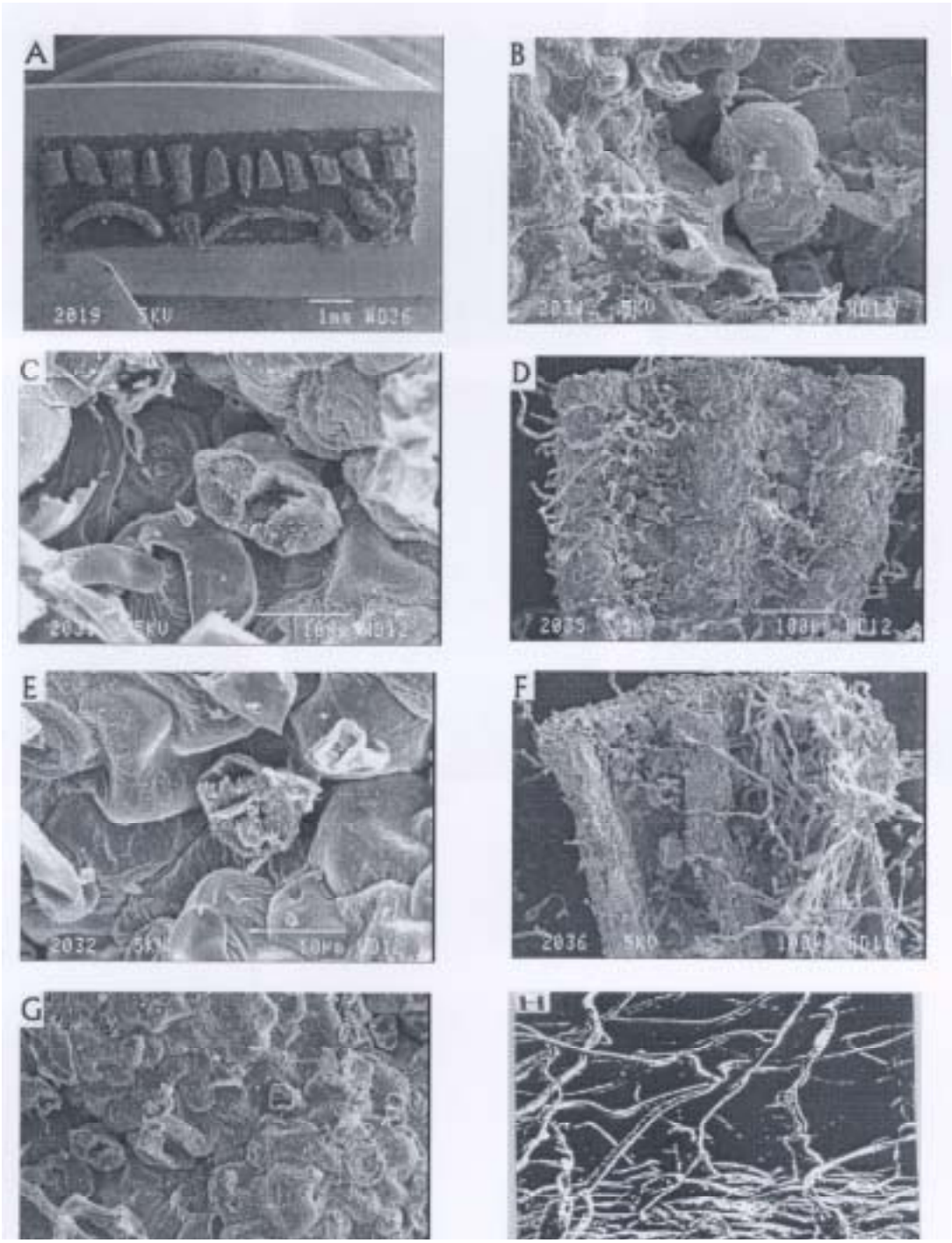


Figure 7.2 SEM dried mounted leaf sections of *H. caespititium*. A. Cross, epidermal and longitudinal sections of tip, middle and base leaf parts mounted for examination. B. Revolute leaf margins and midrib of leaf. C. Abaxial leaf surface revealing bifid nature of trichomes. D. Leaf revealing relationship between revolute margins, glandular trichomes and midrib. E. Torn cuticle subtends stalked basal cells. F. Sparsely distributed trichomes. G. Base cells enchored in epidermal cells. H. High magnification of abaxial surface revealing the morphology of the non-glandular trichomes.

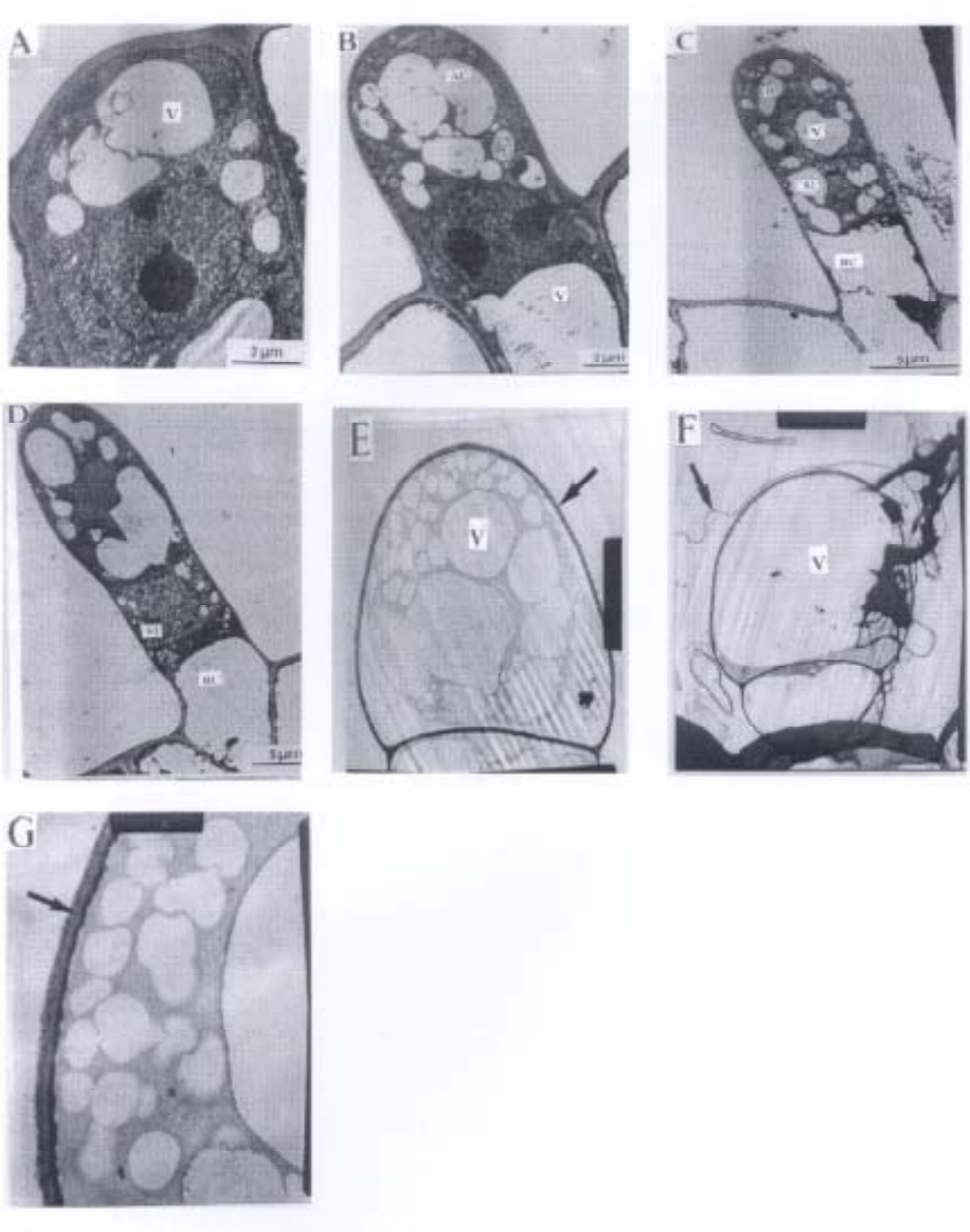


Figure 7.3 Transmission electron micrographs of various stages in the development of the glandular hairs of *H. caespititium.* A. Unicellular stage: papillate outgrowth of epidermal cell representing glandular hair initial. Note that the cytoplasm is dense with an apparent lack of chloroplasts. B. Two-celled glandular hair stage, consisting of an apical cell (AC) and a basal cell (BC). C. Three-celled glandular hair stage, consisting of a head (H), stalk cell (SC) and a

vacuolated, elongated basal cell (BC). D. Four-celled glandular hair stage, consisting of a head (H), two stalk cells (S1 and S2) and a vacuolated, elongated basal cell (BC). E-G. Light micrographs of longitudinal sections of trichomes of *H. caespititium*. E. Oval shaped head. Note smooth cuticle (arrow). F. Globular head. Note that the cuticle has raptured to release a secretory product (arrows). G. Initiation of cell wall and cuticle prior to secretion (arrow).

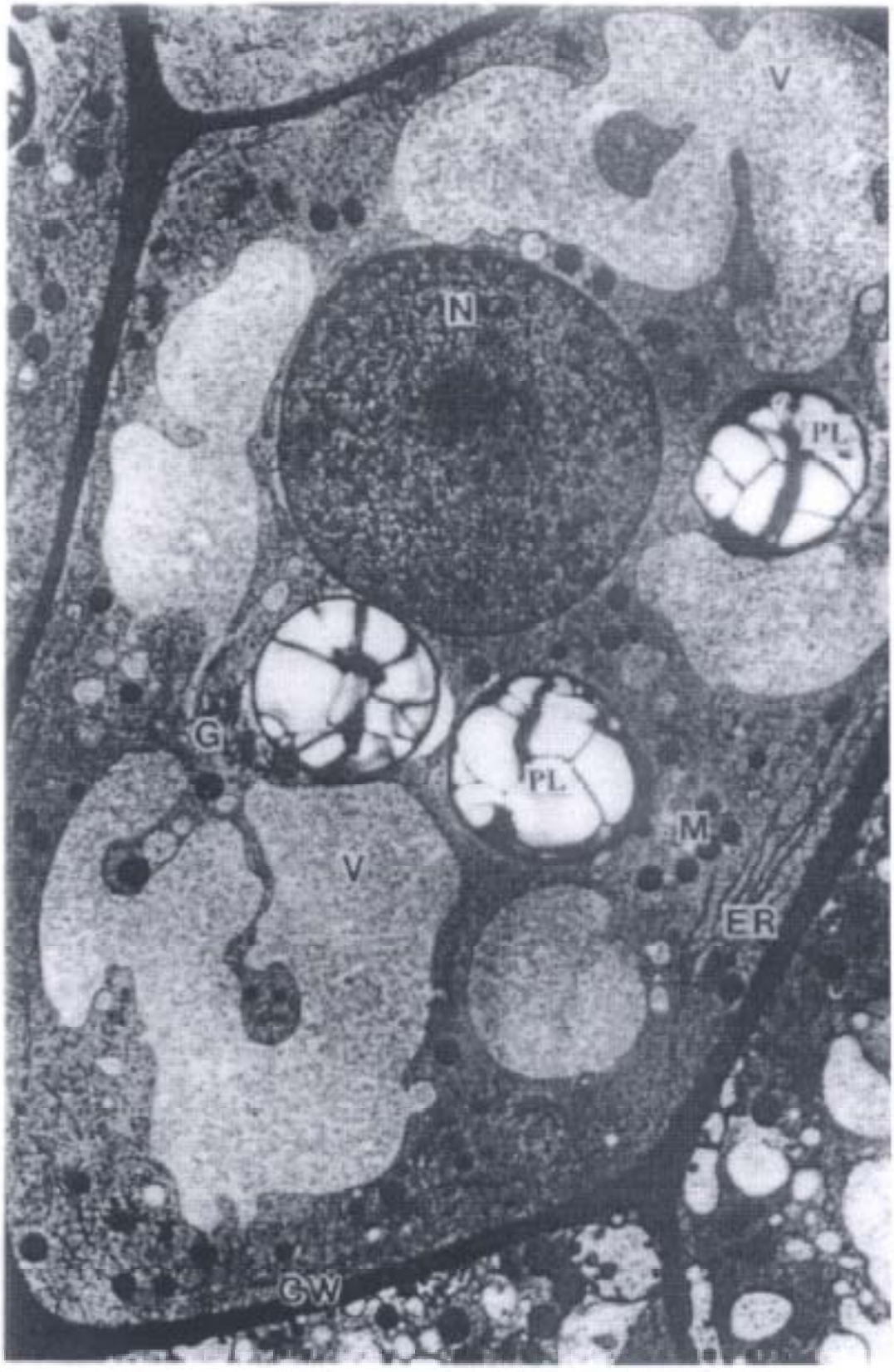

Figure 7.4 Ultrastructure of secretory trichome cell of *H. caespititium.* Cross section of secreting cell. Scale bar 2.5 m. CW= cell wall; ER= endoplasmic reticulum (part of endomembrane system). Note that the proximity of the ER to the cell wall may probably serve to export secretory products to neighbouring cells or secretory cavity; GA= Golgi apparatus; M= mitochondria; N= large nucleus with nucleolus; PL= degenerated plastid; V= vacuole.

A carefully cleaned (de-indumented) abaxial and adaxial leaf surface reveals a striated cuticle devoid of a wax covering. Epidermal cells are isodiametric and polygonal (Figure 7.1F). Glandular trichomes are bifid (Figure 7.2C, 2E and 2G) and glabrous (Figure 7.2 B, 2D and 2F). In this species, trichomes are sparsely distributed between the midrib and revolute margins (Figure 7.2D and 2F ). Single guard-cell stomata occur in *H. caespititium* (Figure 7.1F). Stomata are limited to the lower surface. Each stoma has a thick and prominent outer stomatal ledge. Stomata are superficial or slightly raised, with narrow but long cuticular rims (Figure 7.1F). Stomatal frequence, size, and numbers of cells per unit area were not considered in this study. Adaxial anticlinal cell walls consist of irregular ridges of varying height and thickness (Figure 7.1 B and D). Periclinal walls are slightly concave (Figures 7.1 B and D). Wax was not found on the leaf surface. Abaxial cell boundaries are indicated by shallow grooves of varying width and depth (Figure 7.1F). The leaf epidermis is characterized by distinct oriented striae (Figures 7.1F and H), an indication of no wax on the leaf surface. Non-secretory trichomes are unicellular and usually taper gradually from base to apex (Figure 7.2C, 2E and 2G). Cells eventually break down to wool forming the dense indumentum. (Figures 7.1A-E and G-H). No secretory pores were observed in the cuticle of the secreting trichomes head cell (Figure 7.3E-G) Ultrastructure of secreting trichomes reveals the presence and concentration of mitochondria, endoplasmic reticulum, golgi bodies, vacuoles, plastids, a comparatively

large nucleus with prominent nucleolus, a dense cytoplasm and the other usual cell components (Figure 7.4).

**7. 4. Discussion**

The glandular hairs originate from papillate outgrowths of a single epidermal cell with a relatively large nucleus (Figure 7.3A). This epidermal cell is delimited from the other epidermal cells by its dense cytoplasm, and from the stoma guard cells, which also possess a dense cytoplasm, by the apparent absence of chloroplasts. The initial glandular hair cell elongates markedly and polarization into apical and basal parts occurs by vacuolization of the basal part of the cell (Figure 7.3B). The first cell division is transverse and gives rise to a vacuolated basal cell and apical cell with dense cytoplasm (Figure 7.3B). The basal cell elongate and does not take part in any further cell division so that the head and two stalk cells develop from the apical cell (Figure 7.3C). A transverse division of the apical cell gives rise to a four celled glandular hair consisting of two stalk cells and a head cell as well as a basal cell with dense cytoplasm (Figure 7.3D). The oval head cell enlarges to a dome shape while the basal cell elongates to anchor the trichome (Figure 7.3B-D)

Secretions of compounds probably occur from the young three-celled stage (Figure 7.3C). The young secreting glandular head cell has a smooth surface (Figure 7.3E) but with the accumulation of compounds in the subcuticular space between the cell wall and cuticle a protrusion is formed on top of the head cell (Figure 7.3E-H). No pores occur in the cuticle (Figure 7.3E-G) , therefore it ruptures to release the secretory product (Figure 7.3F). The accumulation process is then repeated since a new cuticle is apparently formed under the ruptured one (Figure 7.3F). Secretion of compounds thus occurs repeatedly in young and old three-celled glandular hairs.

Observations of the repeated secretion of compounds, despite the absence of pores in the cuticleand the rupture of the cuticle with secretions, led to the conclusion that a new cuticle must be formed repeatedly during secretion. In *Inula viscosa*, where lipids are produced continuously throughout the life of the hair, materials are secreted directly through the cell

wall, without the obstacle of the cuticle, once the cuticle has ruptured (Werker and Fahn, 1981). Evidence of formation of a second cuticle is also seen in Figure 3F and G. In the literature no evidence of similar observations in this species and possibly genus could be found and this phenomenon may have implications in the formation of the cell wall and cuticle.

The preceding observations indicate that microscopic features of the leaf may be useful in identifying plants or leaf fragments of *H. caespititium* which are otherwise indistinguishable. Taxa can be separated from each other by a combination of other characters such as a stomatal index, epidermal cell size and pubescence. The isodiametric cells of a species distinguishes it from other taxa of the genus and more importantly, from varieties to which it is most closely related (Olowokudejo, 1990). Trichome density in *H. caespititium* constitutes an important distinguishing feature. The absence of wax on the cuticle can be readily distinguished on by its striated pattern. An artificial identification key may be constructed based on observations made with the SEM and TEM allowing separation of taxa.

These distinguishing epidermal features may be of taxonomic significance because they are reasonably constant in this investigated species. The reliability of epidermal characteristics as taxonomic indicators varies from one group of plants to another. While Mueller (1966) and Cutler and Brandham (1977) have shown that these characters are under strong genetic control, and therefore little affected by the environment, Stace (1965) and Dilcher (1974) indicate that the characters vary according to environmental conditions. The tall order, however, lies in the scanning of the entire genus to verify or achieve this. It can however, be assumed that most of the genetically determined leaf differences are the result of natural selection and are related to a particular combination of (and perhaps trade-off between) various functions. Some properties of the leaf may even have been formed in response to evolutionary pressures different from those encountered by the extant plant (Kerstiens, 1996).

Trichome secretory cells characterized by a dense cytoplasm indicate a well-developed endomembrane system (ES) (Figure 7.4) (Campbell, 1999). The ES includes the nuclear envelope, endoplasmic reticulum (ER), Golgi apparatus (GA), mitochondria, various kinds of vacuoles and the plasma membrane related to the ER and other internal membranes (Figure 7.4). The ER manufactures membranes and performs many other biosynthetic functions.

Trichome secretory cells are rich in smooth ER (Figure 7.4), a standard feature that fits the functions of these cells (Campbell, 1999). The smooth ER functions in diverse metabolic processes, including synthesis of lipids, metabolism of carbohydrates and detoxification of drugs and poisons (Campbell, 1999). Enzymes of the smooth ER help detoxify drugs and poisons. Detoxification involves adding hydroxyl groups to drugs (*H. caespititium* has three hydroxyl groups), making them more soluble and easier to flush from cells (Campbell, 1999). The smooth ER is possibly the location for phloroglucinol synthesis in secretory trichomes.

Alcohol and many other drugs induce the proliferation of smooth ER and its associate detoxification enzymes (Campbell, 1999). This in turn increases tolerance to the drug, meaning that higher doses are required to achieve a particular effect, such as sedation. Also, because some of the detoxification enzymes have relatively broad action, the proliferation of smooth ER in response to one drug can increase tolerance to other drugs as well (broad spectrum). Hence drug abuse, for example, may decrease the effectiveness of certain antibiotics and other useful drugs.

Many types of specialized cells in trichomes secrete various proteins produced by ribosomes attached to the rough ER. Oligosaccharides are covalently bonded to carbohydrates in the rough ER. The ER membrane expands and can be transferred in the form of transport vesicles to other components of the ES (Campbell, 1999).

The GA (Figure 7.4) finishes, sorts and ships cell products. It is a centre of manufacturing, warehousing, sorting and shipping (Campbell, 1999). Products of the ER are modified, according to supply and demand, stored and then sent to other destinations. GA is specifically extensive in trichome cells. The GA has a distinct polarity (*cis* and *trans* faces), with the membranes of cisternae at opposite ends of a stack differing in thickness and molecular composition. The GA removes some sugar monomers and substitutes others, producing oligosaccharides and other compounds (Campbell, 1999).

*H. caespititium* is a mat-forming herb (Figure 1.1), subject to infection by a diversity of soil borne pathogenic viruses, bacteria and fungi that have the potential to damage tissues and even kill the plant. It seems to have a defence system that prevents infection and counters pathogens that do manage to infect the plant.

The first line of defence is the physical barrier of the plant's outer cover, the epidermis of the primary plant body. In addition, *H. caespititium* has a well-developed indumentum of dead non-secreting trichomes. The indumentum undoubtedly plays a significant role in the protection of the aerial parts of the plant. The first line of defence, however, is not impenetrable. Viruses, bacteria and the spores and hyphae of fungi can enter the plant through natural openings in the epidermis such as stomata. Once a pathogen invades, the plant mounts a chemical attack as a second line of defence that kills the pathogens and, prevents their spread from the site of infection. This second line of defence is enhanced by the plant's inherited ability to recognize certain pathogens (Harborne, 1992). This explains the narrow range in activity of some of the plant extracts.

## 7.5 Conclusion

*H. caespititium*'s defence is based on both physical and chemical factors. Physical defence against herbivores are readily appreciated: tough epidermis, cuticular deposits and indumentum. Defence may be purely strategic, as in this case by growing close to the soil

and by vegetative reproduction under the soil surface. Nevertheless, chemical defences are also very important provided by toxins and repellent substances of one type or another within the plant itself. These toxins have had and continue to have a key role in protecting the plant from overgrazing. The ER is possibly the localization of phloroglucinol synthesis in the trichomes of *H. caespititium*. In the leaf and epidermal studies of *H. caespititium*, the most useful morphological and anatomical characters are: the indumentum, the cuticle, palisade ratio, pattern of anticlinal walls, density and type of trichomes. These characteristic features have been used in framing a key to the species to make it possible to identify the species either in the vegetative or fragmented state (Ogundipe, 1992).

The indumentum and cuticle have jointly a wider perspective of structure-function relationships, namely, to form a mechanical barrier against penetration by fungal hyphae and insect mouth-parts; reduce the uncontrolled loss of water and apoplastic damage; and protect the tissues from mechanical damage. In addition, they act as an accommodation compartment of exudate compounds on the leaf surface.

The structure of the cuticle suggests that its main function is to act as a medium for plant signals perceived from insects or microbes arriving on the leaf surface, hence the absence of wax . The range of chemical compounds located on the surface and found to be involved in processes such as host recognition and herbivore deterrence is bewildering. Some of them, such as natural pesticides and the antimicrobial compounds found in *H. caespititium* also may be exuded by the biseriate glandular trichomes. Probably the majority, however, reach the surface after diffusing across the cuticle (Kerstiens, 1996).

The wide diversity of functions fulfilled by the appendages of the leaf of *H. caespititium* indicate the possible structural links or overlaps between different functions including secretion of antimicrobial compounds. It is not surprising that not a single obvious conflict between the requirements of different functions, forcing a trade-off, could be identified.

The way the leaf and its appendages have evolved seems to be extraordinarily well suited to playing many different roles at a time.

Leaf morphological and ultrastructural trichome studies in the genus *Helichrysum* are rare or non-existent. An intensive morphological and ultrastructural study of the genus to construct a leaf key to the species which would make it possible to identify the species either vegetatively or in a
fragmented state is imperative for purposes of easy identification, classification and comparative studies.

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