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Early Development of the Secretory Cavity of Peltate Glands in *Humulus lupulus* L. (Cannabaceae)

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Early development of the secretory cavity of chemically fixed peltate glands in *Humulus lupulus* L. showed secretions with different densities, light, gray and dark, in the cytoplasm of disc cells and in the periplasmic space adjacent to the developing secretory cavity. Secretions were detected in the disc cell wall and subsequently in the developing secretory cavity under the subcuticular wall of the sheath. Light and gray secretions in the cavity possessed a membrane-like surface feature. Secretions were in contact with the irregular inner surface of the cuticle. Secretions contributed to the thickening of the cuticle, whereas the membrane-like surface feature contributed to a network of *Cannabis* striae distributed throughout the cuticle. This study supports an early development and organization of the secretory cavity in *H. lupulus*, parallel to those in *Cannabis*, and may represent common features for lipophilic glands in angiosperms.

Keywords: Development; Electron Microscopy; *Humulus*; Peltate Gland; Secretion; Secretory Cavity.

Introduction

Peltate glands on bracts of pistillate flowers in *Humulus lupulus* L. (hop) form a noncellular secretory cavity containing secretions such as alpha- and beta-acids and other flavoring compounds used in the brewing industry. Studies link the gross morphology of these glands with the accumulation of alpha-acid (Maeda, 1977; Menary and Doe, 1983). Initial studies on gland development and histochemistry report the presence of numerous secretory vesicles in a secretory cavity formed under the cuticle (Mahlberg and Kim, 1992). Secretions were first detectable in the secretory cells after the appearance of vesicles in the secretory cavity (Menary and Doe, 1983; Oliveira and Pais, 1988; 1990). However, characteristics of the secretory cavity, the process whereby secretions pass into the secretory cavity and their possible role in the cavity remain unclear.

Mature peltate glands of *H. lupulus* resemble the capitate glands of *Cannabis* which consist of a discoidal tier of secretory cells subtending a large non-cellular secretory cavity. In *Cannabis* tangential partitioning of the outer disc cell wall results in the formation of an intrawall cavity bounded externally by a sheath consisting of a subcuticular wall and cuticle. Secretions formed in the cytoplasm of disc cells pass through the plasma membrane to accumulate in the periplasmic space between the plasma membrane and the cell wall, and subsequently migrate through the disc cell wall into the secretory cavity. Upon emerging in the cavity, the secretions are enveloped with a membrane-like surface feature of unknown character derived from components of the disc cell wall. Other components in the wall, described as fibrillar matrix and particulate material, are also present in the secretory cavity, and seem to pass from the wall into the secretory cavity. The secretory vesicles, upon contacting the subcuticular wall, migrate through this wall to contact the inner surface of the cuticle, where the vesicle contents became incorporated into a thickening cuticular layer. Upon the fusion of vesicles with the cuticle, their membrane-like surface feature contributes to a network of channels, or striae, that permeate the cuticle (Kim and Mahlberg, 1991; 1995; 1997a; Mahlberg and Kim, 1991; 1992).

Previous studies on glands of other plants report the presence of secretions in a secretory cavity enveloped with a sheath consisting of only a cuticle (Ascensao *et al.*, 1995; Bosabalidis and Tsekos, 1984; Fahn, 1988; Schnepf, 1974), although recently the sheath was reported to include subcuticular wall material comparable to that for *Cannabis* (Ascensao *et al.*, 1995; 1997).

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We hypothesize that the process of secretion into the secretory cavity and the structural organization of the secretory cavity are similar in lipophilic glandular trichomes among angiosperm taxa. Correlated studies of secretory cavity development should include taxa from diverse families of flowering plants. The secretory cells of lipophilic glands secrete terpenes and specialized secondary products distinctive to a taxon, which accumulate in the secretory cavity (Hammond and Mahlberg, 1994; Kim and Mahlberg, 1997b, c; 1999). Some secretions, such as volatile monoterpenes, are common to glands in many taxa and contribute to plant scents and other functions; whereas other specialized substances are stored in, or exuded from, a gland with as yet unclear functions (Kim and Mahlberg, 1997c; 1999; Wagner, 1991).

In this paper, we examine the early phase of secretory cavity development in *Humulus* including: (1) the localization and accumulation of secretions in the disc cell, cell wall and secretory cavity, and (2) the possible fate of secretory products in the gland.

Materials and Methods

H. lupulus plants, a variety of Willamette, were grown to flowering under greenhouse conditions. Glands on bracts of pistillate flowers at different developmental stages were fixed in a solution containing 6.6% glutaraldehyde and 3% dimethyl sulfoxide, in a sodium cacodylate buffer (pH 7.2) overnight at 4°C. Fixed tissues were washed in 50 mM cacodylate buffer and post-fixed with aqueous 2% osmium tetroxide vapor for 2.5 h at room temperature to control post-fixation to a gray-black color and minimize over-reaction. Tissue pieces were immersed in buffer in one well of a spot plate along with a drop of osmium tetroxide in an adjacent well. The spot plate was maintained in a covered Petri dish in the hood for the fixation period. Tissues were rinsed several times in deionized water to remove the buffer, dehydrated in a graded ethanol-acetone series and embedded in Spurr's resin. Thin sections were cut on an LKB-IV ultramicrotome with glass or diamond knives and stained with uranyl acetate followed by lead citrate. Sections were examined and photographed with a JEOL 1010 transmission electron microscope at 60 kV.

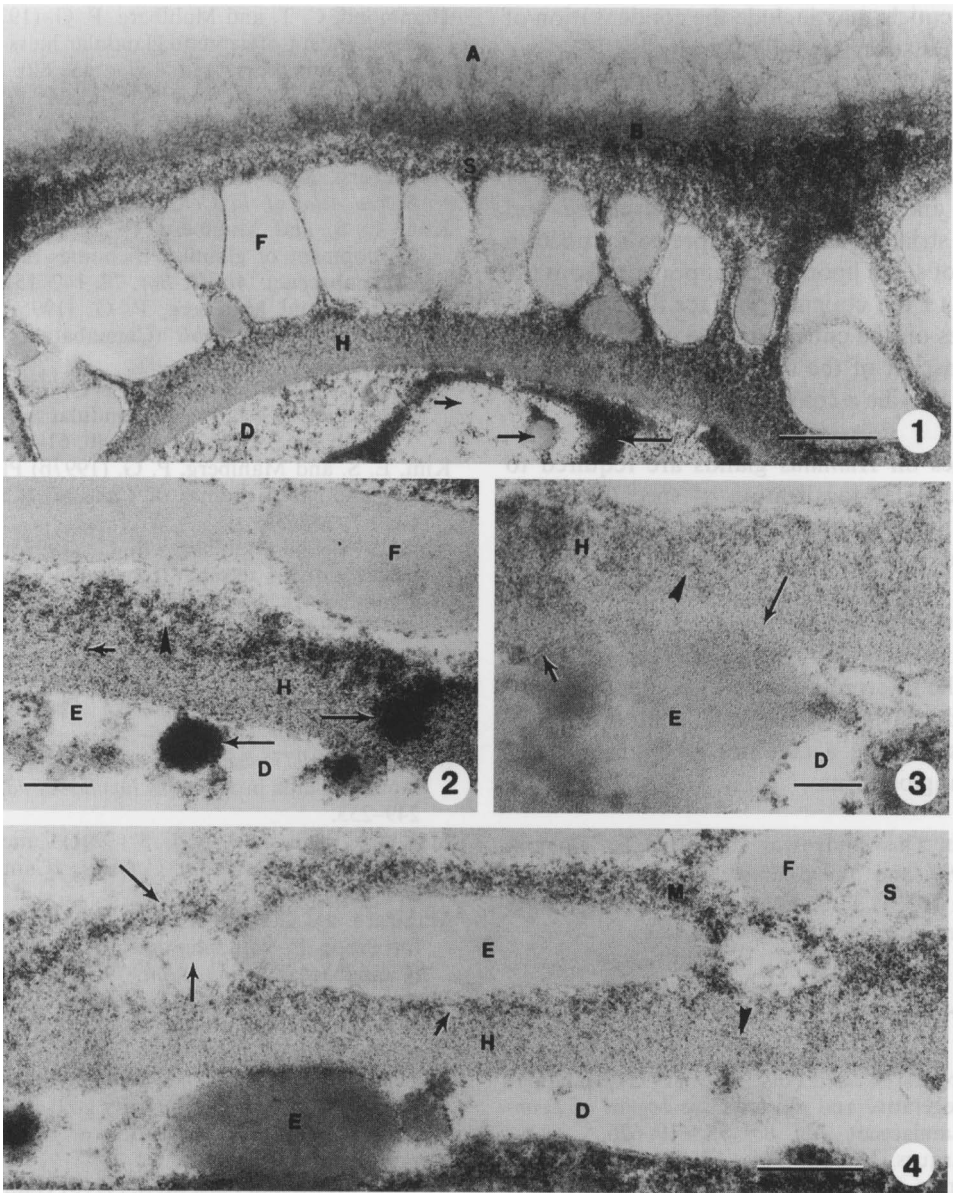
Results

Presence of secretions in the disc cells Disc cells at an early phase of secretory cavity formation had a dense cytoplasm and contained secretions with different densities in the region facing the secretory cavity (Fig. 1). These secretions appeared as light, gray and black masses. Gray secretions were typically round in shape, whereas black and light masses were either round or irregular in shape. Secretions with different densities and sizes appeared as dense masses in the region adjacent to the cell wall (Figs. 2 and 3). Gray areas in contact with the wall appeared continuous with it (Fig. 3). In other views, a faint demarcation was evident where a gray secretion contacted the wall; but elsewhere along the contact surface the secretion appeared continuous with the wall. Light secretions were evident adjacent to the wall as well as in the wall (Fig. 2). Dark secretions were evident adjacent to the cell wall (Figs. 1 and 2). These secretions contained particulate materials, which were also present in the cell wall.

Secretions in the wall Gray secretions were also present in the wall. They differed in size, but often appeared as voluminous, somewhat horizontally compressed bodies in the wall (Fig. 4). Light secretions were distributed throughout the wall, and appeared as small hyaline areas in the dense wall (Fig. 4). They occurred individually in the wall or were aggregated adjacent to or around the periphery of other secretions (Figs. 4 and 6). When aggregated together, their surface feature was not clear in the central region of the cluster (Fig. 4). Particulate material was present throughout the inner and outer regions of the wall, but appeared most abundant in the outer region (Figs. 5 and 6). Elsewhere along the wall surface aggregates of fibrillar matrix and particulate material extended into the secretory cavity (Fig. 5).

Secretory cavity Secretion masses with different sizes occupied the small secretory cavity (Fig. 1). The membrane-like surface feature was most evident around enlarged secretions where it appeared as a single electron-dense line separating the secretions from other secretory vesicles, fibrillar matrix and particulate material in the cavity (Fig. 5). The homogeneous contents of adjacent vesicles were confluent where the surface feature appeared as a constriction across their midregions (Fig. 5). Light vesicles accumulated around the gray vesicles in the secretory cavity (Figs. 5 and 6). The fibrillar matrix was present throughout the secretory cavity as short segments (Figs. 5 and 7). Materials with a similar appearance were present in the disc cell wall (Fig. 6). They were not evenly distributed in the cavity. Particulate material associated with the wall matrix extended from the wall into the secretory cavity (Figs. 5 and 6). It consisted of dark particles among other components in the wall, and was also evident throughout the cavity (Fig. 5). It was evident too on the membrane-like surface feature of the vesicles (Fig. 6).

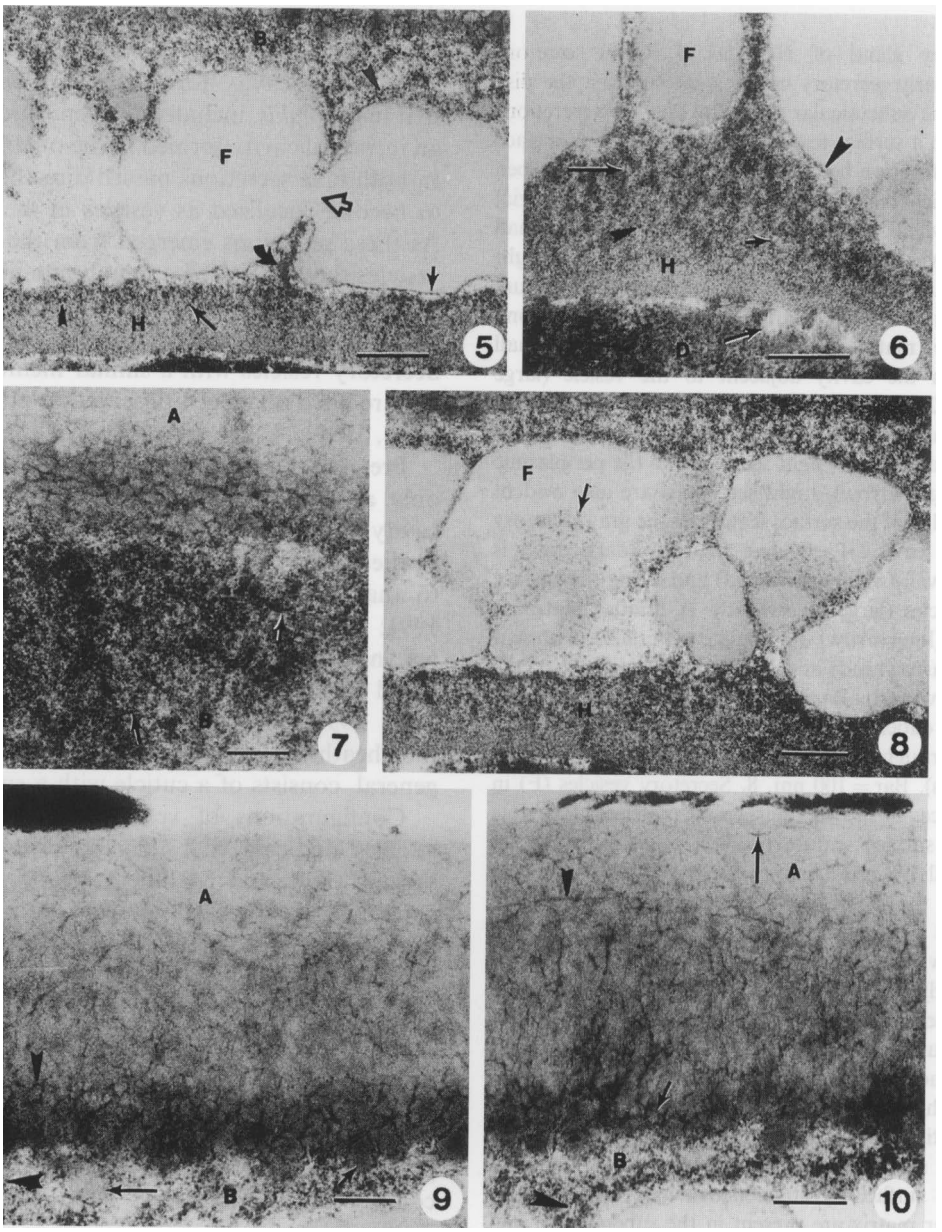
Sheath A zone of fibrillar matrix and particulate material was present under the cuticle and interdigitated into the irregularities of the inner cuticular surface (Figs. 1 and 7). Particulate materials and short segments of fibrillar matrix were evident in the subcuticular wall (Figs. 9 and 10). Light and gray vesicles were also present in the subcuticular wall and contributed to the irregular character of this surface (Figs. 9 and 10).



Figs. 1–4. Secretory gland of *Humulus*. **1.** Early secretory cavity (S) with a row of large gray secretory vesicles (F) positioned between the disc cell wall (H) and the subcuticular wall (B) under the cuticle (A). Light, gray and dark secretions (short, medium and long arrows, respectively) are present in the disc cell (D). Bar = 200 nm. **2.** Dark secretion (long arrows) occur in the disc cell (D) and in the disc cell wall (H). Particulate material is present elsewhere in the wall (short arrow). A light secretion (E) is present adjacent to the disc cell wall as well as in the wall (arrowhead). Bar = 100 nm. **3.** Gray secretion (E) pressed against the disc cell wall (H). A faint demarcation line between it and the wall is evident at the left (short arrow), but not at the right where it appears fused to the wall (long arrow). Light secretions are present in the wall (arrowhead). Bar = 100 nm. **4.** A large somewhat compressed gray secretion (E) is present in the disc cell wall (H). A light secretion is also present in the wall (short arrow). Wall material above the secretion (E) will enter the secretory cavity (S) as fibrillar matrix (M), also evident above the symbol M. Secretions (E) are present between the plasma membrane and cell wall (D). A gray secretory vesicle (F) is evident in the secretory cavity (S). Particulate material (long arrow) is distributed throughout the cell and also in the periplasmic space. A short segment of fibrillar matrix is present in the wall (arrowhead). Bar = 200 nm.

The membrane-like surface feature was present around some of the vesicles that contacted the lower surface of the cuticle, but was no longer detectable on other vesicles (Fig. 9). Residual portions of the membrane-like surface feature appeared as dark lines in the proximity of vesicles associated with the thickening cuticle. These portions were contiguous with other dark lines in the lower region of the cuticle (Figs. 9 and 10).

The dark lines formed an anastomosed network throughout the cuticle (Figs. 9 and 10). Long lengths of dark lines extended toward the outer cuticular surface



(Fig. 10). They became reoriented from more or less round features surrounding the vesicles to irregular elongated features in the cuticle. Lengths of this network were oriented in various directions, including horizontal, vertical and diagonal, relative to the surface of the cuticle.

Irregular, homogeneous dark masses of material were present on the cuticular surface of glands possessing a secretory cavity (Figs. 9 and 10), but were absent from this surface prior to the formation of the cavity (data not shown). These masses sometimes appeared partially imbedded in the cuticle (Fig. 9). The network of dark lines permeated the cuticle to the vicinity of these surface masses, although the lines were less dense than those near the inner surface of the cuticle (Figs. 9 and 10).

Discussion

Disc cells of the secretory glands of *Humulus*, like those of *Cannabis*, in a longisectional view possess a polar orientation in that the portion facing the secretory cavity differs physically from the lateral and basal regions (Hammond and Mahlberg, 1978; Kim and Mahlberg, 1991). This orientation of glands is evident for *Humulus* and other taxa, as illustrated but not discussed in other reports (Ascensao *et al.*, 1995; Bosabalidis, 1990; Thomson and Healey, 1984). This study illustrates: (1) the passage of secretions and particulate materials through the plasma membrane and outer cell wall, (2) the increased thickening of this wall and the cuticle, and (3) that the release of wall materials into the secretory cavity supports the inter-

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Figs. 5–10. Secretory gland of *Humulus*. **5.** Gray secretory vesicles (F) in an early secretory cavity stage between the disc cell wall (H) and the subcuticular wall (B). The gray secretions are surrounded with a surface feature (short arrow). The surface feature shows a perforation between two secretory vesicles (open arrow) where a vesicle has become constricted by wall material (curved arrow) or where two secretory vesicles have fused. Small gray vesicles are evident above the large vesicles. Small light vesicles are present along the outside edge of the surface feature (below short arrow). A fibrillar matrix is present in the wall (long arrow). Particulate material is present in the wall (small arrowhead) and in the cavity adjacent to the vesicle (large arrowhead). Bar = 200 nm. **6.** Thickened region of the disc cell wall (H) showing numerous hyaline areas of light secretions in the cell wall (short arrow) and light secretions in the periplasmic space (medium length arrow). Light secretions are also evident along the outside edge of the surface feature of the gray secretory vesicles (F) in the cavity. Particulate material (dark dots) is evident in the cell wall (small arrowhead) and along the surface feature of the vesicles (large arrowhead). A fibrillar matrix is present in the wall (long arrow). Particulate materials are present in the wall (small arrowhead) and in the cavity adjacent to the vesicles (large arrowhead). Bar = 200 nm. **7.** Subcuticular wall area (B) under the cuticle (A) showing short lengths of fibrillar matrix (arrows) throughout the wall and abundant particulate material (dark dots). Bar = 100 nm. **8.** Secretory vesicles (F) in the early secretory cavity showing particulate material (dark dots at arrow) on the surface feature (grayish area) as shown in surface view. Small hyaline areas are present in the wall and accumulate along the outer surface feature of the vesicles, as evident around symbol H in the wall. Bar = 200 nm. **9.** Vesicle (long arrow) surrounded with a surface feature, in the subcuticular wall adjacent to the inner irregular surface of cuticle (A). Another smaller vesicle is directly above this vesicle and in contact with the cuticle. Other vesicles (short arrow) are fused with the lower surface of the cuticle. Numerous other vesicles are darkly outlined with the former surface feature which contributes to striae in the cuticle (small arrowhead). Black material (top, left) on the outer surface of the cuticle may be a secretion, transported to the surface by striae (small arrowhead). Short segments of fibrillar matrix are present in the subcuticular wall (large arrowhead). Bar = 100 nm. **10.** Cuticle (A) showing the distribution of striae throughout the cuticle. They are evident from the inner surface (short arrow), where vesicles fuse to the cuticle, nearly to the outer surface (long arrow) subjacent to dark material on the cuticle surface. Striae show various orientations; even some horizontally positioned long lengths of striae (arrowhead) occur in the cuticle. Short segments of fibrillar matrix are present in .the subcuticular wall (large arrowhead). Bar = 100 nm.

pretation that this plasma membrane area adjacent to the secretory cavity differs functionally from the membrane area elsewhere around these cells. It is appropriate, therefore, to designate the plasma membrane area facing the secretory cavity as the apical domain, and the remaining surface area as the basolateral domain. This terminology for plasma membrane surfaces parallels designations describing these regions in animal secretory cells (Hollenberg *et al.*, 1989).

Secretory cavity formation in *Humulus*, paralleling that in *Cannabis*, included the deposition of secretions in an intrawall cavity formed in the outer wall of disc cells. In both taxa secretions passed directly through the wall to become localized as vesicles in the secretory cavity. As these secretions emerged from the wall as secretory vesicles they possessed a membrane-like surface feature of unknown composition derived from wall materials (Kim and Mahlberg, 1991; Mahlberg and Kim, 1992). Secretory vesicles with a similar membrane-like surface feature have now been observed in other tax (Ascensao *et al.*, 1997).

Previous studies on the glands of *Humulus* report only a cuticle delimiting the sheath of the secretory cavity (Oliveira and Pais, 1988; 1990), whereas our studies show the sheath of *Humulus* to be similar to that described for *Cannabis* (Kim and Mahlberg, 1991; 1995). A subcuticular wall can be observed, but was not described, for the sheath in other reports on gland organization (Bourett, *et al.*, 1994; Duke and Paul, 1993; Sacchetti, *et al.*, 1999). We propose that the sheath of the lipophilic glands of angiosperms, in general, consists of a cuticle with a subcuticular wall.

Configurations of vesicles, such as those of light vesicles, indicate that they may fuse to form larger vesicles (Kim and Mahlberg, 1995). Alternatively, the constrictions observed on vesicles can be interpreted as a fission phenomenon. Both phenomena may occur. Vesicles with different densities may reflect different contents. The presence of a surface feature may function to control fusion in that different lipophilic compounds may be confined to specific vesicles by a surface feature which somehow regulates their composition. Studies on the composition of different secretory vesicles are pertinent to understanding the localization of secreted components (Oliveira and Pais, 1988; Sacchetti *et al.*, 1999).

The function of dark secretions and their particulate material is unknown, but particulate materials become distributed throughout the secretory cavity. Similar materials were reported in the secretory cavity of *Cannabis* (Kim and Mahlberg, 1991; 1995; Mahlberg and Kim, 1992). Dark secretions may be the source of materials for the formation of the membrane-like surface feature.

The contents of the secretory vesicles function to thicken the cuticle by apposition along the inner cuticular surface. A similar appositional pattern of cuticle thickening was reported for *Cannabis* (Mahlberg and Kim, 1991). Secretory vesicles fusing to the cuticle were typically small, in contrast to the large secretory vesicles abundant in the secretory cavity. Factors controlling vesicle size, including fusion or fission, require further study. The incorporation of the vesicle

content into the cuticle may include the condensation of some contents to a solid state (Cutler *et al.*, 1982).

Upon fusion of the vesicle contents with the cuticle, the membrane-like surface feature contributes to the strial network extending throughout the cuticle. This network was first reported for the developing cuticle of *Cannabis* (Kim and Mahlberg, 1995; Mahlberg and Kim, 1991). The strial network is interpreted to function in the transport of some lipophilic components from the secretory cavity to the cuticular surface. The accumulated dark masses on the cuticle surface may be derived from such a transport of the secretory cavity contents. A strial network may be a common structural feature of lipophilic glands.

Further studies on *Humulus* glands are required to identify the subcellular localization of specialized secretory products, such as humulone and lupulone. The specialized secretory product, tetrahydrocannabinol, was shown to accumulate in the membrane-like surface feature, the cuticle, and the walls in the glands of *Cannabis* (Kim and Mahlberg, 1997; 1999). This result supports the interpretation that the deposition of specialized products into structural components of lipophilic glands may be a common phenomenon for these glands in angiosperms.

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