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Secretory Vesicle Formation in the Secretory Cavity of Glandular Trichomes of *Cannabis sativa* L. (Cannabaceae)

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The disc cell wall facing the secretory cavity in lipophilic glands of *Cannabis* was studied for origin and distribution of hyaline areas, secretory vesicles, fibrillar matrix and particulate material. Secretions evident as light areas in the disc cell cytoplasm pass through modified regions in the plasma membrane and appear as hyaline areas in the cell wall. Hyaline areas, surrounded with a filamentous outline, accumulate near the wall surface facing the secretory cavity where they fuse to form enlarged hyaline areas. Fibrillar matrix is related to and may originate from the dense outer layer of the plasma membrane. This matrix becomes distributed throughout the wall material and contributes in part to the composition of the surface feature of secretory vesicles. Thickening of the cell wall is associated with secretions from the disc cells that facilitates movement of hyaline areas, fibrillar matrix and other possible secretions through the wall to form secretory vesicles and intervesicular materials in the secretory cavity. The outer wall of disc cells in aggregate forms the basilar wall surface of the secretory cavity which facilitates the organization of secretory vesicles that fill the secretory cavity.

Keywords: Basilar Wall; Cannabaceae; *Cannabis*; Cryofixation; Gland; Secretory Cavity.

Introduction

Glandular trichomes of *Cannabis sativa* L. consist of a group of specialized cells, disc cells, associated with the production of diverse secretions that accumulate in a large intrawall secretory cavity. During development the cell wall under the cuticular sheath partitions tangentially to initiate the formation of this cavity. Upon wall partitioning the outer wall portion remains associated with the cuticle to form the subcuticular wall of the secretory cavity whereas the remaining inner portion constitutes the disc cell wall facing the secretory cavity (Hammond and Mahlberg, 1973; 1977; 1978; Kim and Mahlberg, 1991; 1995; 1997a; Mahlberg and Kim, 1992). Recently, a similar organization was described for lipophilic glands in *Leonotis* and *Humulus* (Ascensao *et al.*, 1997; Kim and Mahlberg, 2000).

Secretions in the disc cells, as observed in *Cannabis*, are interpreted to pass through the plasma membrane and cell wall facing the secretory cavity as hyaline areas and accumulate as secretory vesicles in the secretory cavity (Kim and Mahlberg, 1997b). As hyaline areas emerge from the wall surface in the cavity they become surrounded with a surface feature. Upon their detachment from the wall suface these new vesicles accumulate in the secretory cavity. Some vesicles adjacent to the sheath penetrate the subcuticular wall to contact the cuticle whereupon their contents contribute to the thickening of the inner cuticular surface. The surface feature of a vesicle that fuses with the cuticle remains as a stria in the thickened cuticle. This stria becomes associated with other striae to form a network of microchannels throughout the cuticle. We interpret these microchannels to function possibly for the release of volatile secretory components from the cuticular surface (Kim and Mahlberg, 1995; Mahlberg and Kim, 1991). Similar striae are reported to occur in the cuticle of *Humulus* (Kim and Mahlberg, 2000).

Analyses of isolated secretory glands show them to contain a complement of lipophilic terpenes and phenols (Hammond and Mahlberg, 1990; 1994; unpublished). An abundance of terpenes is reported to occur in *Cannabis* (Ross and ElSohly, 1996). Terpenes are reported to be synthesized in plastids (Gleizes *et al.*, 1983) and, there-

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fore, we interpret their synthesis to occur in the numerous specialized plastids, lipoplasts, in the disc cells of the gland (Kim and Mahlberg, 1997b). Studies show that the secretory cavity contains cannabinoids, or terpenophenolic compounds, which include tetrahydrocannabinol, THC (Lanyon *et al.*, 1981; Turner *et al.*, 1978). Other studies, using an electron microscopic probe prepared from a monoclonal antibody for THC, show THC to be abundant in the cavity and localized on the surface feature surrounding each vesicle, in the cuticle over the secretory cavity as well as in the cell wall; it was not detected in the secretory vesicles (Kim and Mahlberg, 1997c; 1999).

The apparent disc cell wall under the secretory cavity thickens during the enlargement of this cavity in association with the accumulation of secretory products. Several distinctive components appear in this wall, including hyaline areas, fibrillar matrix and particulate material, after initiation of the secretory cavity. Hyaline areas and fibrillar matrix appear to be associated with secretory vesicle formation, whereas the particulate material becomes widely distributed throughout the wall and between secretory vesicles in the cavity (Kim and Mahlberg, 1991; Mahlberg and Kim, 1991).The origin of these components and their fate in the wall are incompletely known.

The disc cell wall facing the secretory cavity is unusual in that it appears to thicken during secretory cavity development and serves as a structure through which secretions from disc cells must pass to enter the cavity. The plasma membrane region subjacent to this wall is also unusual because the secretory components must pass through it to enter the cell wall before they can accumulate in the cavity. Therefore, we hypothesize that 1) the plasma membrane along the disc cell wall surface becomes modified to allow for passage of secretions, 2) the plasma membrane contributes physical components to the assemblage of materials in the disc cell wall, and 3) hyaline areas in the wall contribute their contents to developing secretory vesicles. In this report we examine the plasma membrane and cell wall region of disc cells facing the enlarged secretory cavity to interpret the origin and development of hyaline areas, secretory vesicles and fibrillar matrix.

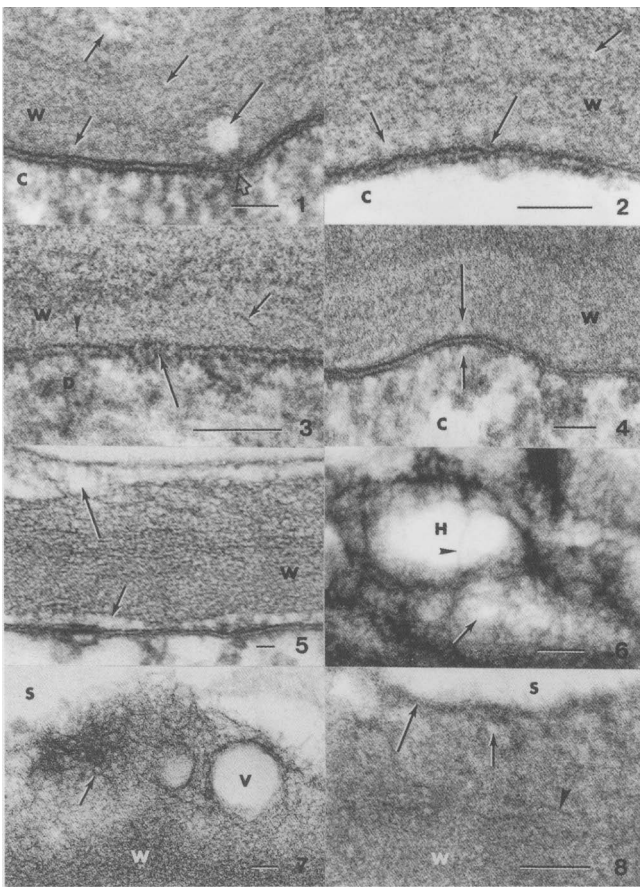
Materials and Methods

Bract segments containing glands of different developmental stages, obtained from pistillate *Cannabis sativa* L. grown under greenhouse conditions (Hammond and Mahlberg, 1973), were fixed and processed by the Balzers high pressure cryofixation and cryosubstitution (HPC-CS) procedure (Hunziker and Schenk, 1984; Kim and Mahlberg, 1997c). Small segments of fresh bracts were placed in brass Swiss Precision planchettes, immersed in 1-hexadecene (Sigma, USA), and immediately transferred to a Balzers HPM 010 for cryoimmobilization under 2100 bar pressure (Hunziker and Schenk, 1984). The samples were then transferred immediately to liquid nitrogen and stored briefly until all samples were ready for cryosubstitution (CS). The planchettes were quickly transferred to sieve-dried methanol containing 3% glutaraldehyde or 3% glutaraldehyde and 1% osmium tetroxide and immediately placed in the Balzers model FSU automated CS instrument. CS proceeded uninterrupted from −90°C to −60°C to −30°C, each for 8 h, and at 0°C for 1 h. Following CS the samples were transferred to fresh sieve-dried methanol over ice, subsequently brought to room temperature, and immediately embedded in Spurr's low-viscosity resin (Spurr, 1969). Thin sections of embedded material were prepared with diamond or glass knives on an ultramicrotome (LKB-V) and stained with 1% uranyl acetate for 10 min followed by 1% lead citrate for 10 min (Reynolds, 1963). Sections were examined and photographed with a transmission electron microscope (JEOL 1010) at 60 kV.

Results

Plasma membrane The bilayered plasma membrane along the cell surface of disc cells facing the secretory cavity was typically appressed against the wall (Fig. 1, at left). The continuity of this membrane interrupted at some positions, particularly where there were light areas in the wall and dense accumulations of cytoplasm in the cell (Fig. 1, at right). At other positions along this membrane the upper layer was constricted where components in the cytoplasm contacted its inner bilayer (Fig. 2). Continuity of the bilayer was incomplete and the intermembrane zone was somewhat dilated where dense components of cytoplasm contacted the membrane (Fig. 3). The organization of the bilayer was incomplete where it was in close proximity to large hyaline areas in the wall near the membrane, although the bilayer character was evident on both sides of the obscured membranous area (Fig. 1). The plasma membrane was intact and appressed against the wall along the lateral and lower wall surfaces of the disc cells where these cells contacted each other or the stipe cell (not shown).

Hyaline areas in wall Small hyaline areas were present in the wall near the plasma membrane (Fig. 1). Similar somewhat larger hyaline areas, about 10-20nm in diameter, were closely arranged in tandem along the wall surface adjacent to the plasma membrane (Figs. 2 and 4). Hyaline areas were also evident throughout the wall (Figs. 1 and 2), They were larger and more abundant in the wall region adjacent to the secretory cavity where they occurred individually or in clusters, or accumulated as a horizontal layer in the wall material adjacent to the secretory cavity (Fig. 5). Large hyaline areas, about 50 nm in diameter, were present in the wall, and some were very close to the plasma membrane (Fig. 1). The filamentous outlines inside the hyaline areas indicated that it was



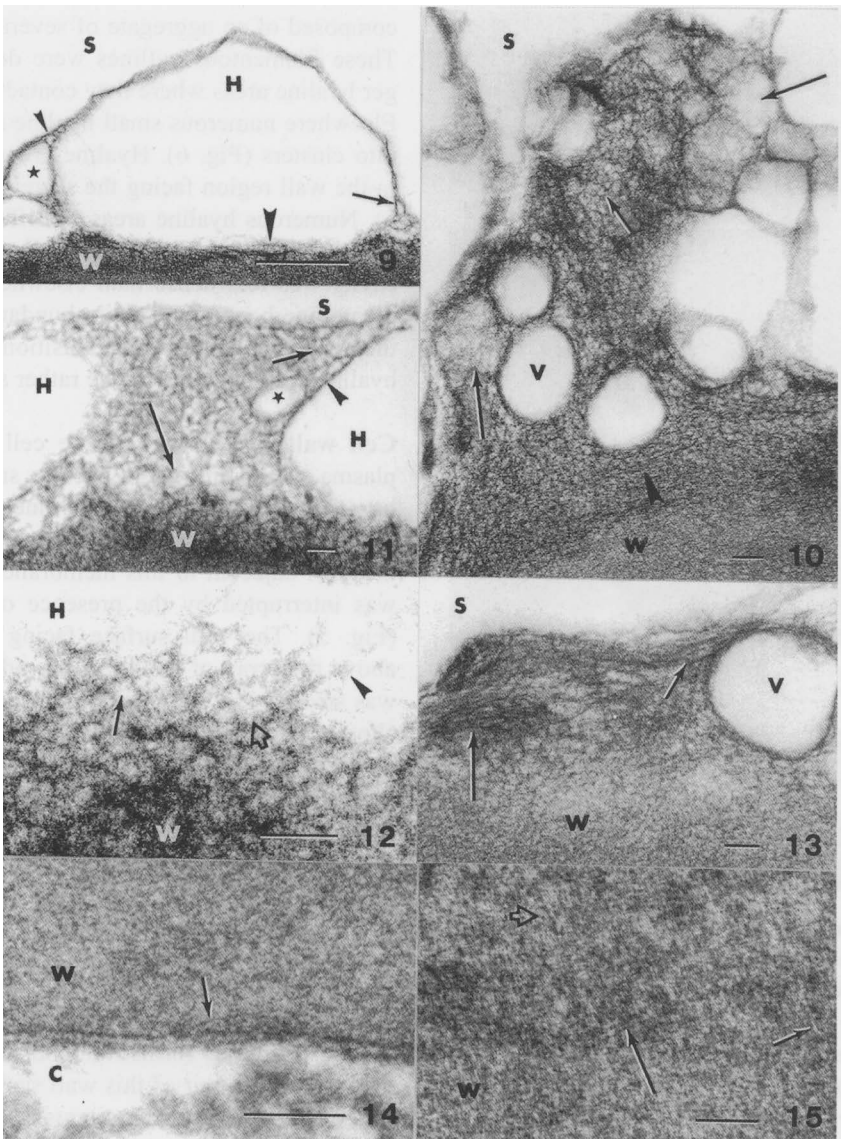
Figs. 1-8. Disc cell wall. **1.** Plasma membrane bilayer with discontinuous sector (clear arrow) adjacent to large hyaline area (long arrow) in wall (W). Small hyaline areas (short arrows) distributed throughout wall. C, secretion product. Bar = 50 nm. **2.** Constriction in plasma membrane (long arrow). Several hyaline areas (short arrows) adjacent to plasma membrane and in wall (above W). C, Secretion product. Bar = 50 nm. **3.** Plasma membrane bilayer incomplete (long arrow) where it contacts dense cytoplasmic components (D) in disc cell. Particulate material adjacent to plasma membrane (arrowhead) and in linear arrangement in wall (short arrow). W, wall. Bar = 50 nm. **4.** Several hyaline areas in wall (long arrow, and to left of arrow) adjacent to plasma membrane. Light zones (short arrow) in cell adjacent to plasma membrane and opposite hyaline area in wall (W). C, secretion product. Bar = 50 nm. **5.** Numerous hyaline areas aggregated in wall (W) along plasma membrane (short arrow) and immediately under (long arrow) secretory cavity; a thin layer of wall material is above these hyaline areas. Bar = 50 nm. **6.** Large hyaline area (H) containing filamentous feature (arrowhead) in wall. Numerous small hyaline areas aggregated into cluster (arrow). Bar = 50 nm. **7.** Wall (W) showing accumulation of hyaline areas (arrow) near surface of secretory cavity (S). Secretory vesicle (V) to right. Bar = 50 nm. **8.** Fibrillar matrix (arrowhead) and hyaline areas (short arrow) in wall among similar components elsewhere in wall (W). Interface between secretory cavity (S) and wall area (long arrow) is diffuse rather than a sharp line, Bar = 50 nm.

composed of an aggregate of several small hyaline areas. These filamentous outlines were detectable between larger hyaline areas where they contacted each other (Fig. 6). Elsewhere numerous small hyaline areas were aggregated into clusters (Fig. 6). Hyaline areas were most abundant in the wall region facing the secretory cavity (Figs. 7 and 8). Numerous hyaline areas of different sizes were accumulated in this region. The wall region adjacent to the cavity was less dense than elsewhere toward the plasma membrane because of their abundance in the region near the secretory cavity. The transition from small to larger hyaline areas appeared to be rather abrupt.

Cell wall The surface of the cell wall adjacent to the plasma membrane was typically smooth, and the membrane was in contact with the inner wall surface (Fig. 1). However, when numerous hyaline areas were present in the wall adjacent to this membrane, its smooth character was interrupted by the presence of these hyaline areas (Fig. 5). The wall surface facing the secretory cavity, above the array of hyaline areas adjacent to this surface, was smooth (Fig. 5). This surface appeared as a thin band above the hyaline areas, and faced a large secretory vesicle in the secretory cavity. At other positions this surface was irregular, or feathery, in its contour (Figs. 7 and 8). Masses of grayish material extended from the wall into the lighter region of the secretory cavity. In some locations the contour of the gray material appeared u- or cup-shaped (Fig. 7). The wall was irregular along the base of the enlarged hyaline area that projected into the secretory cavity (Fig. 9).

Material along the wall surface facing the secretory cavity sometimes formed mounds that were raised above the general contour of this wall surface (Figs. 10 and 11). Large mounds were voluminous. Clusters of small hyaline areas were partially or completely surrounded with a dark outline. Other bordered round areas contained filamentous of numerous small hyaline areas. Yet other round areas surrounded with a dense border had a clear interior (Fig. 10). In addition to these bordered areas, these mounds contained fibrillar matrix, particulate material and small hyaline areas. Other quantities or masses of secretions were localized between enlarged hyaline areas that projected into the secretory cavity (Fig. 11). This mass appeared to be suspended between two large hyaline bodies and the large secretory vesicle above it. The mass contained a small secretory vesicle surrounded with a surface feature (Fig. 11, asterisk), and other small hyaline areas. Numerous hyaline areas were present adjacent to the dense region of the cell wall.

Secretory vesicle formation Numerous hyaline areas were aggregated near the surface of the wall facing the secretory cavity (Fig. 5). A thin layer of wall material separated them from the secretory cavity. The hyaline



Figs. 9-15. Disc cell wall. **9.** Enlarged hyaline area (H) arched against secretory vesicle in secretory cavity (S). Surface feature of hyaline area extends around arch (short arrow) to dense wall (W). Fibrillar matrix (large arrowhead) evident among small hyaline areas in wall. Surface feature (small arrowhead) of large secretory vesicle in cavity covers several small vesicles at and above asterisk in thin layer of intervesicular zone continuous with wall. Bar = 10 nm. **10.** Mound of material from wall (W) projecting into secretory cavity and surrounded at extreme right and left by larger secretory vesicles in cavity (S). Numerous hyaline areas are aggregated into clusters (short arrow). Some structures (long arrows) possess filamentous outlines of hyaline areas and are surrounded by a somewhat dense border. Other structures (V) have no hyaline areas evident within them but possess a dense border. Fibrillar matrix (arrowhead) evident in mound above wall (W). Bar = 50 nm. **11.** Sheet of material projecting from dense wall (W) into secretory cavity between hyaline areas (H) and secretory vesicle in secretory cavity (S). Secretion material (in front of short arrow) extends into intervesicular zone between vesicle in secretory cavity and adjacent hyaline area (H at right). Surface feature (large arrowhead) present in portion of enlarged hyaline area at right. Small hyaline areas (long arrow), small secretory vesicle with surface feature (asterisk) and other unidentified materials are present in sheet. Bar = 50 nm. **12.** Small hyaline area (short arrow) at base of enlarged hyaline area (H). Cluster of small hyaline areas that appear to have fused at central region of contact (clear arrow). Filamentous outline (arrowhead) extend into secretory area of large hyaline area (H). Particulate material, evident as particles, distributed among numerous small hyaline areas in wall (W). Bar = 50 nm. **13.** Quantity of fibrillar matrix (long arrow) in wall (W) along edge of secretory cavity (S). Fibrillar matrix (short arrow) extends to and partly envelops secretory vesicle (V). Bar = 50 nm. **14.** Fibrillar matrix (short arrow) in contact with bilayer of plasma membrane facing wall (W). Light zone of secretions (C) present in cell adjacent to plasma membrane. Bar = 50 nm. **15.** Parallel (long arrow) and curved (short arrow) segments of fibrillar matrix in wall (W). Small hyaline areas (small light areas) abundant in wall. Abundant particulate material (clear arrow) in wall. Bar = 50 nm.

areas under this layer of wall material were larger in size than hyaline areas in other regions of the wall. Elsewhere along the wall area were arched configurations that protruded from the wall into the secretory cavity (Fig. 9). The arch consisted of a thin layer of material lifted from the outer region of the wall. The arch was sandwich-like: its outer surface was associated with the large secretory vesicle in the secretory cavity (Fig. 9, S); its inner surface was the component of an enlarged hyaline area, or developing secretory vesicle (Fig. 9, H). The inner and outer surfaces of the arch were visible at the lower right in the figure where they appear as an inverted Y. Similarly, the sandwich nature was evident at the left along the arch where they, again, form an inverted Y (Fig. 9, arrowhead). At these Y locations the two surface features separate and surround a small secretory vesicle.

The surface along the wall, which formed the base of the arch (Fig. 9, long arrow), was irregular with grayish material projecting from the wall into the clear central area of the developing secretory vesicle. A surface feature was absent along this region of the wall surface. The sandwich-like character of the arch region was evident in an enlargement of such an intervesicular zone (Fig. 11). Materials from the wall region extended into the arch (Fig. 11, short black arrow) bounded to the outside by the surface feature of the secretory vesicle in the secretory cavity (Fig. 11, S) and to the inside by the surface feature of the enlarged hyaline area (Fig. 11, H). These materials included small hyaline areas and particulate material, among other components, and were in contact with the wall. A small vesicle (Fig. 11, asterisk), bounded by a surface feature, was among components in the material. The surface feature of the hyaline area was not evident along the region where the hyaline area contacted the wall; rather, gray material extended into the clear portion of the hyaline area. A mirror image of the sandwich organization was evident at the right for the upper vesicle and the enlarged hyaline area, but the image was not a median section for the surface features of these structures (Fig. 11).

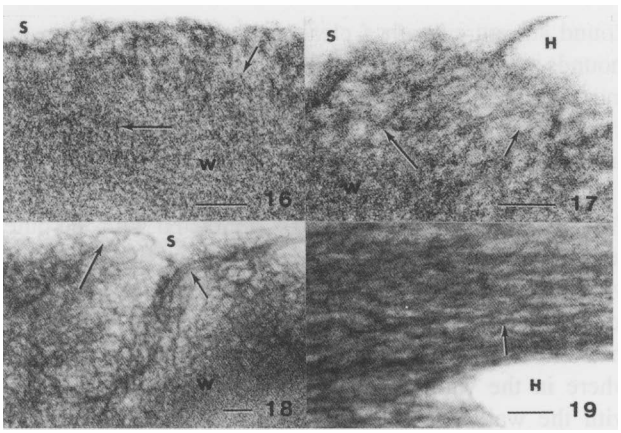
Microscopic enlargement of the wall region facing an enlarged hyaline area showed the presence of numerous small hyaline areas in the wall and at its surface adjacent to the hyaline area (Fig. 12). The small hyaline areas were evident as light areas surrounded by gray to nearly black material of unknown composition. Some of these small hyaline areas were aggregated together and lacked gray material at their point of contact (Fig. 12, short arrow). Hyaline areas in the denser region of the wall were small (Fig. 12, near W) whereas those near the clear region were typically larger in size (Fig. 12, long arrow). Other hyaline areas appeared to lack a portion of their surface feature where they faced the light cavity. Elsewhere the gray portions extended into the cavity. Some of the gray portions were more or less u-shaped or irregular in outline. Round structures with a clear center were present in the mounds of material along the wall surface (Figs. 7, 10, and 13). They possessed a dense peripheral boundary from which gray material often extended a short distance into the clear area.

Fibrillar matrix Fibrillar matrix contacted with the outer dense band of the plasma membrane (Fig. 14). One fibril was about 45 nm long. These fibrils, here oriented at an acute angle to the membrane, appeared relatively thinner than the dark band of the bilayer. Fibrillar matrix elsewhere in the wall was oriented more or less in parallel with the wall surface and was present between hyaline areas and other wall components (Figs. 14 and 15). Elsewhere in the wall the segments of fibrillar matrix were oriented approximately at right angles to the plasma membrane (Fig. 16). Near the surface of the wall material facing the secretory cavity this matrix was evident as individual or small clusters of fibrils (Fig. 17). Some of the fibrils were evident as curved structures (Fig. 18). At some positions along the wall surface they occurred as bundles of fibrils, very much like a brush heap (Fig. 13). These fibrils were in contact with the dense boundary of secretory vesicles along the wall surface facing the secretory cavity (Fig. 13). Fibrillar matrix accumulated in large quantities at positions along the wall surface facing the secretory cavity (Fig. 19). Although it was possible to recognize the fibril character of this material it was difficult to resolve structural detail or length of individual fibrils even in such masses.

Particulate material Particulate material was present throughout the wall material as individual particles and was present near the plasma membrane as well as near the wall surface facing the secretory cavity (Figs. 2, 3, and 16). At some positions these particles were closely spaced into short linear configurations (Fig. 3). These particles also occurred among small hyaline areas in the mounds and thin sheets of materials that extended above the wall (Fig. 11).

Discussion

Capitate glands of plants are reported to contain a diverse array of secondary products, including terpenes, phenols, cannabinoids, and other lipophilic and non-lipophilic compounds which are accumulated or secreted by these structures (Duke *et al.*, 2000; Hallahan, 2000). Some plants contain compounds unique to a genus, such as *Cannabis*, which is known to contain the terpenophenolic cannabinoid compounds. These specialized compounds have been shown to occur in the secretory cavity of the gland (Kim and Mahlberg, 1997c; 1999; Lanyon *et al.*, 1981; Turner *et al.*, 1978). Other compounds, including



Figs. 16-19. Disc cell wall. **16.** Fibrillar matrix (long arrow), several segments oriented at nearly right angle to wall surface (W) facing secretory cavity (S). Particulate material (short arrow) widely distributed in wall. Bar = 50 nm. **17.** Fibrillar matrix (long arrow and elsewhere) oriented at acute angles to wall surface (W) among hyaline areas (short arrow) near secretory cavity (S) and enlarged hyaline area (H). Particulate material (dark particles) and hyaline areas (light areas) present in wall. Bar = 50 nm. **18.** Curved fibrillar matrix segments (short arrow) among hyaline areas (light areas) throughout wall (W) and near surface (long arrow) facing secretory cavity (S). Bar = 50 nm. **19.** Portion of fibril (arrow) in a large mass of fibrillar matrix near wall surface adjacent to hyaline area (H). Bar = 50 nm.

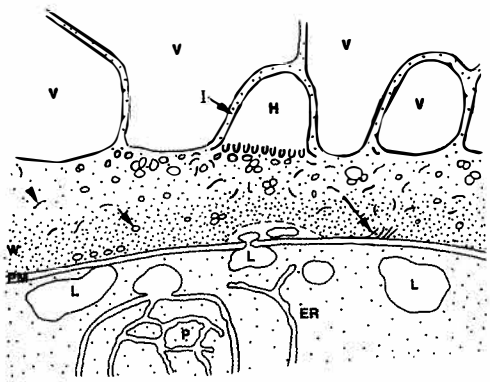


Diagram 1. Disc cell wall in *Cannabis* gland. Light areas (L) in disc cell pass through plasma membrane (PM) to form hyaline areas (short arrow) in wall (W) and accumulate at wall surface facing secretory vesicles (V) in the secretory cavity. Small hyaline areas (U-shaped) fuse with enlarging hyaline area (H). Fibrillar matrix (long arrow) is in contact with plasma membrane. Fibrillar matrix in wall (arrowhead) contributes to surface feature formed around enlarging hyaline area (H) developing into new secretory vesicle (V at right). Intervesicular zone (I) is continuous with disc cell wall materials. Plastid (P) with content of light area and endoplasmic reticulum (ER) are components of disc cell.

terpenes and phenols, also occur in the glands of *Cannabis* (Hammond and Mahlberg, 1990; 1994). Localized modifications of the plasma membrane, such as the development of a diffuse area or a constriction at very localized sites in the bilayer, are interpreted as loci where substances synthesized in the disc cell can pass through the membrane to enter the cell wall. These loci have typically dense cytoplasmic materials associated with them that may include components being secreted from the cell into the wall. A similar phenomenon of penetration of the plasma membrane by substances associated with plastids in the disc cells was also reported recently (Kim and Mahlberg, 1997b). Lipophilic terpenes, reported to be synthesized in plastids (Gleises *et al.*, 1983), may include such secreted substances that can penetrate the plasma membrane to enter the wall subjacent to the secretory cavity of a glandular trichome.

The presence of light areas in the cytoplasm adjacent to the plasma membrane and the occurrence of hyaline areas in the wall, such as in a tandem arrangement along this membrane, support an interpretation that the light areas pass through modified regions of the plasma membrane to become evident as hyaline areas in the cell wall (Diagram 1). Small hyaline areas can aggregate to form larger areas in which their filamentous outlines remain evident within the aggregate. The appearance of larger hyaline areas, lacking filamentous outlines within them, supports an interpretation that these larger areas formed upon fusion of the small hyaline areas. The filamentous outlines appear to become incorporated into the surface feature of the enlarged hyaline area. The continual incorporation of small hyaline areas into one of large size along the wall surface facing the secretory cavity results in an enlarged hyaline area, or incipient secretory vesicle, that protrudes much like an arch into the secretory cavity (Diagram 1). As enlargement of the hyaline area continues it lifts a thin layer of wall material into the secretory cavity in the form of an arch. The inner surface of the arch is the surface feature of the hyaline area whereas the outer surface is the surface feature of a much larger secretory vesicle in the secretory cavity. The inner surface feature of the enlarged hyaline areas extends to the dense wall region but initially does not extend across its base. Numerous small hyaline areas along the dense wall region fuse with the enlarged area to contribute both their content and filamentous outline to the incipient secretory vesicle. When the surface feature of the incipient vesicle becomes extended across its base to form a continuous surface around this vesicle it then represents a new secretory vesicle in the secretory cavity (Diagram 1). The production of secretory vesicles is a continuing process along this wall surface. The formation of new secretory vesicles along the common or basilar wall surface of the secretory cavity progressively displaces those formed previously that become positioned elsewhere in the secretory cavity (Diagram 2). The large secretory vesicles in the secretory cavity have been re-

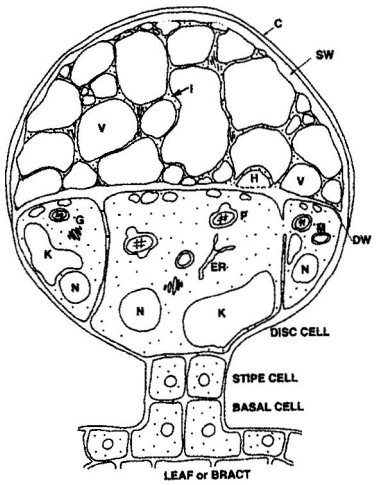


Diagram 2. *Cannabis* gland supported by stipe and basal cells on leaf or bract. Secretions from plastids (P) in disc cell accumulate as small light areas (irregular circles) near plasma membrane of cell wall under secretory cavity, and pass through this membrane into disc cell wall (DW) to become hyaline areas that accumulate into an enlarged hyaline area (H) at wall surface under secretory cavity. An enlarged hyaline area separates from wall to form a secretory vesicle (V) in secretory cavity. Secretory vesicles throughout secretory cavity are separated from each other by intervesicular zone (I) continuous with materials of the basilar wall. Small secretory vesicles pass through subcuticular wall (SW) and their contents thicken inner surface of cuticle (C). Plasmodesmata (lines through wall) and large pores occur in lateral walls of disc cells. N, nucleus; ER, endoplasmic reticulum; G, Golgi apparatus; M, mitochondrion; P, plastid; K, vacuole.

ported to result from the fusion of adjacent vesicles (Kim and Mahlberg, 1995; Mahlberg and Kim, 1992). The irregular outlines of numerous small hyaline areas and their filamentous outline at the base of an incipient vesicle suggest that they are dissociating and adding their contents to the vesicle (Diagram 1). The filamentous outlines of these hyaline areas appear to contribute to the surface feature of the incipient vesicle. The factors that control loading of secretions into an incipient vesicle, vesicle size and vesicle detachment from the wall surface remain to be determined.

The intervesicular zone contains substances outside of the secretory vesicles and pervades the secretory cavity (Diagram 2). Its composition is incompletely known although it would include secretions in addition to the evident fibrillar matrix and particulate material. Substances within this zone become distributed throughout the secretory cavity and, along with secretory vesicles, may include components incorporated into the sheath (Kim and Mahlberg, 1995; Mahlberg and Kim, 1991). The presence of large bordered features consisting of aggregates of small hyaline areas, of similar features filled with filamentous outlines and of other bordered features with a clear content supports an interpretation that the hyaline areas had fused and their filamentous outlines had become incorporated into peripheral border of these structures to form a secretory vesicle. The apparent fusion of hyaline areas parallels the report that secretory vesicles may also fuse (Kim and Mahlberg, 1995; 2000).

The association of fibrillar matrix with the enlarged hyaline areas and secretory vesicles near the wall surface supports an interpretation that the matrix contributes to the formation of the surface feature of secretory vesicles. Its diverse orientation in the wall, aggregation into fibrillar masses, presence in the intervesicular zone and association with the thickening of the subcuticular wall indicate that it is somehow transported throughout the secretory cavity (Kim and Mahlberg, 1995). Fibrillar matrix is also reported in the secretory cavity in glands of *Humulus* (Kim and Mahlberg, 2000). Fibrils were observed to be associated with the outer bilayer of the plasma membrane. Their presence in contact with this bilayer could be a result either 1) of the fibrils originating from the membrane, or 2) of existing fibrils somehow moving to and contacting the bilayer surface. We propose that the fibrils originate from the outer bilayer of the plasma membrane because of their abundance and distribution throughout the cell wall and intervesicular zone as fibrillar matrix. No other source for generation of fibrillar matrix is evident in the cell wall or contents of the secretory cavity. There is no evidence for fibril synthesis in the disc cell and the subsequent movement of fibrils through the plasma membrane to enter the cell wall. Thus, the plasma membrane directly or indirectly may be associated with synthesis of the fibrils from structural subunits derived from the disc cells. The possible association of the fibrillar matrix with the outer bilayer of the plasma membrane may be unique to the glandular trichome. However, the phenomenon would complement the observations of the presence of microtubules along the inner surface of this membrane inside living cells in general (Traas, 1990). Microtubules are present in the glandular disc cells of *Cannabis*, but they are associated with this membrane along the lateral walls; they were not detected along the region of this membrane under the secretory cavity (Kim and Mahlberg, unpublished).

The wall of the disc cells appears electron dense close to the plasma membrane, but the inclusion of hyaline areas, fibrillar matrix and other possible secretions, makes it difficult to distinguish its boundary toward the secretory cavity. Some inclusions contribute to the mounds and sheets of substances that project into the secretory cavity and contribute to the intervesicular zone. We interpret these substances to be secretions that differ from the contents of hyaline areas, but their composition is unknown at present. The plasma membrane region of the disc cells facing the secretory cavity provides for passage of greater

quantities of secretions than regions elsewhere in the cell. This regional difference was first described for the secretory cells in glands of *Humulus* (Kim and Mahlberg, 2000). They described the plasma membrane region facing the secretory cavity as the apical domain of this membrane, in contrast to regions along the lateral and basal walls of secretory cells that they designated as the basolateral domain of the membrane. These terms, derived from similar designations applied to secretory cells in animals (Hollenberg *et al.*, 1989), appropriately describe regional differences for secretory activity of the plasma membrane in cells of capitate glands.

In summary, the secretory cavity resembles a large dome with a basal wall surface composed of the disc cell walls in aggregate facing the cavity. As secretory vesicles emerge along the basilar wall surface, and enlarge by the addition of the contents of adjacent hyaline areas, they become surrounded with a surface feature and subsequently detach from the basilar wall to become individual secretory vesicles in the secretory cavity. An intervesicular zone, which contains secretions deposited in the secretory cavity by the disc cells, separates individual secretory vesicles from each other and permeates the entire secretory cavity. Further studies on lipophilic glands in other families will provide data on whether the pattern of gland development observed in *Cannabis* is similar throughout the Angiosperms.

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