

SECRETORY VESICLE FORMATION IN GLANDULAR TRICHOMES OF *CANNABIS SATIVA* (CANNABACEAE)¹

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Formation of secretory vesicles in the noncellular secretory cavity of glandular trichomes of *Cannabis sativa* L. was examined by transmission electron microscopy. Two patterns of vesicle formation occurred during gland morphogenesis. 1) During initial phases of cavity formation small hyaline areas arose in the wall near the plasma membrane of the disc cell. Hyaline areas of elongated shape and different sizes were distributed throughout the wall and adjacent to the secretory cavity. Hyaline areas increased in size, some possibly fusing with others. These hyaline areas, possessing a membrane, moved into the cavity where they formed vesicles. As membraned vesicles they developed a more or less round shape and their contents became electron-dense. 2) During development of the secretory cavity and when abundant secretions were present in the disc cells, these secretions passed through the wall to accumulate as membraned vesicles of different sizes in the cavity. As secretions emerged from the wall, a membrane of wall origin delimited the secretory material from cavity contents. Vesicles released from the wall migrated in the secretory cavity and contacted the sheath where their contents permeated into the subcuticular wall as large or diffused quantities of secretions. In the subcuticular wall these secretions migrated to the wall–cuticle interface where they contributed to structural thickening of the cuticle. This study demonstrates that the secretory process in glands of *Cannabis* involves not only secretion of materials from the disc cell, but that the disc cell somehow packages these secretions into membraned vesicles outside the cell wall prior to deposition into the secretory cavity for subsequent structural development of the sheath.

Lipophilic glandular trichomes form secretory vesicles that accumulate in a distended noncellular secretory cavity (Hammond and Mahlberg, 1977, 1978). Secretory vesicles have been described only for glands of *Cannabis*, although they have been detected in glands of other plants (Oliveira and Pais, 1990).

The secretory cavity in *Cannabis* arises by the splitting of the outer wall of disc cells to form a noncellular compartment into which disc cells deposit precursors for sheath development and other poorly understood functions. The disc cell wall facing the cavity releases wall matrix which contributes to the thickening of the subcuticular wall during gland development (Kim and Mahlberg, 1991). Secretions accumulated in the cavity as membraned vesicles are utilized to construct a thickened cuticle on the sheath (Mahlberg and Kim, 1991). The mechanism whereby wall matrix and secretions are formed and released under the control of the disc cells is unknown and, therefore, important to an understanding of gland development. Few data are available on the formation of the wall matrix and secretions.

In this study we examine the development of secretory vesicles in the secretory cavity of the *Cannabis* gland. Our objectives are to determine 1) vesicle origin during secretory cavity development and enlargement, 2) the movement of secretion products through the disc cell wall, and 3) the relationship of the vesicles to cuticle development. As a result of this study we describe an unusual process for the delimitation of secretory vesicles by a membrane originating in the cell wall.

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MATERIALS AND METHODS

Procedures employed in this study of glandular trichomes on the bracts of pistillate *Cannabis* plants grown under greenhouse conditions were described previously (Kim and Mahlberg, 1991; Mahlberg and Kim, 1991).

RESULTS

Formation of secretory vesicles during early development of secretory cavity—Secretory cavity initiation was detected by the loosening of wall materials in the outer wall of the disc cells (Mahlberg and Kim, 1991). This wall loosening process, evident as an electron-light space in the wall, occurred within the outer region of the wall and was not detected in anticlinal walls of disc cells (Fig. 1). As the area of the secretory cavity enlarged, various materials associated with the wall were deposited into the cavity. Wall matrix, interpreted to be structural components of the wall, separated from the wall surface to be deposited in the cavity. Electron-light hyaline areas, which represented distinctive components in the wall, also were deposited in the developing secretory cavity (Fig. 2).

Hyaline areas of very small size typically were detected along the inner edge of the wall in the proximity of the plasma membrane (Fig. 3). Other hyaline areas, but larger in size, were evident in the wall toward its surface facing the secretory cavity (Figs. 3, 4). At positions in the wall distant to the plasma membrane these hyaline areas often were elongated in form and corresponded to the areas associated with the early phase of secretory cavity initiation (Fig. 2). Numerous hyaline areas were present in the wall during these early stages of secretory cavity development. A progression in size for these areas was evident in the wall with small-sized areas adjacent to the plasma membrane and progressively larger-sized hyaline

areas throughout the wall to the surface of the secretory cavity.

The plasma membrane showed folds along its surface in the vicinity of the small hyaline areas in the wall (Fig. 3). Also, small vesicles were evident outside the plasma membrane (Fig. 4). Small quantities of material interpreted to be secretory product (black) occasionally were evident in the cytoplasm of the disc cell (Fig. 4).

Hyaline areas frequently appeared in a tandem arrangement in the wall suggesting their aggregation into elongated areas and apparent movement through the wall (Fig. 5). Dark strands traversed the hyaline areas. A strand may represent residual wall material at a location where two hyaline areas had fused to form their tandem arrangement.

Hyaline areas arose along the entire surface of the outer wall of the disc cell including the juncture region between adjacent disc cells (Fig. 6). In such regions, because of the thickness of the wall, the progressive increase in size of hyaline areas was evident. Small-sized hyaline areas were present in the region where the walls of both cells contacted each other and where hyaline areas of progressively larger size were evident toward the secretory cavity. The hyaline areas appeared to be bounded by a membrane.

Flattened hyaline areas extended into the secretory cavity from the wall (Fig. 7). This particular hyaline area appeared to be an aggregation of several joined into a tandem arrangement, whereas others in the wall were smaller in size (Figs. 2, 4).

Vesicles of different sizes were evident in the secretory cavity (Figs. 3, 5, 7). Typically, these vesicles possessed a dense content and were surrounded by a distinct membrane (Figs. 5, 6). As hyaline areas entered the secretory cavity from the wall their contents became electron-dense (Fig. 7). Dense contents appeared to have formed in that portion of the hyaline area that projected into the secretory

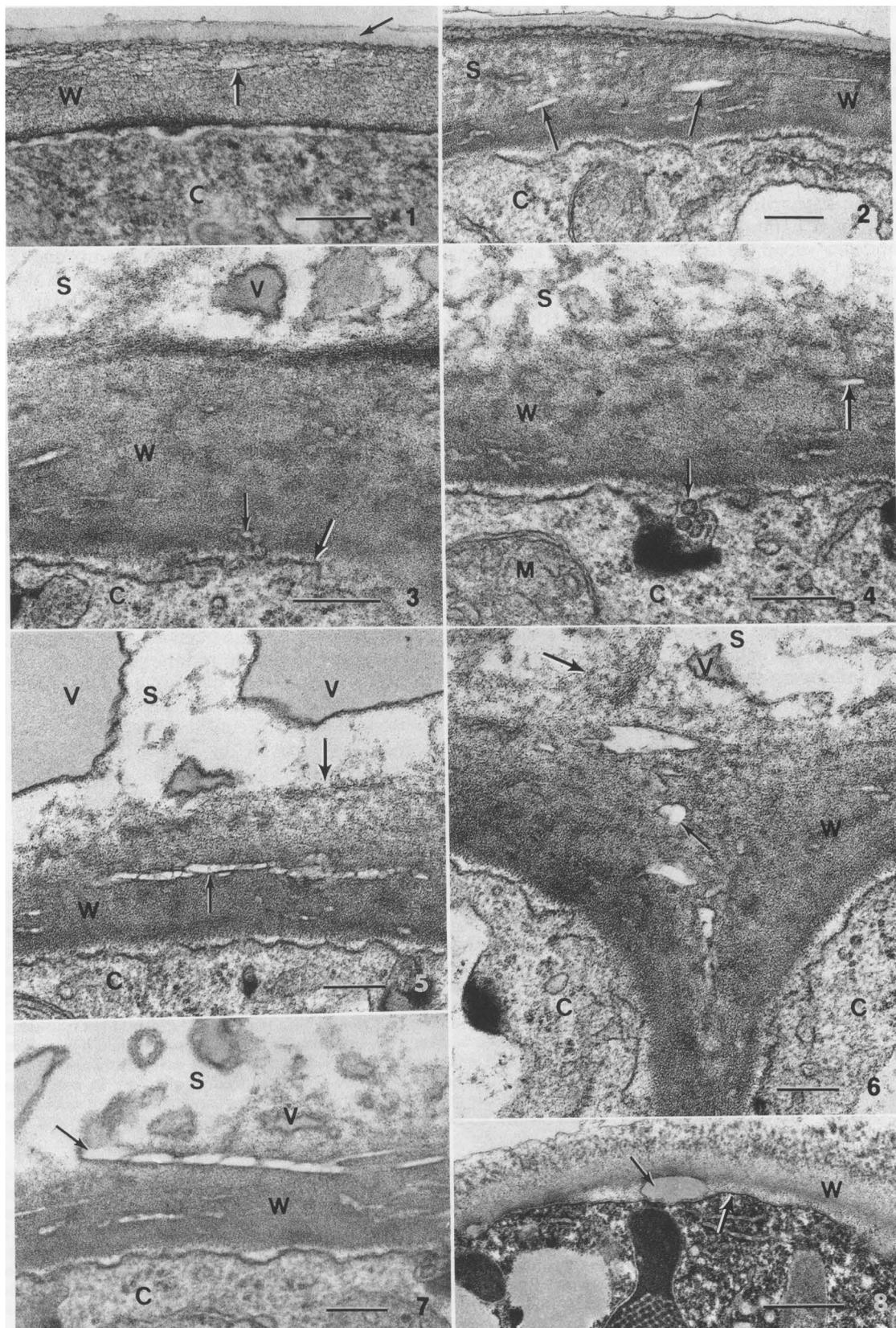
cavity, while contents of that portion of the hyaline area in the wall were electron-light in appearance (Fig. 7). The several hyaline areas above this elongated one appeared to possess electron-dense contents and will move into the cavity along with the elongated one (Fig. 7). Flattened hyaline areas, upon being deposited in the secretory cavity, were interpreted to become filled with electron-dense secretory materials and formed a more or less round shape (Figs. 3, 5–7).

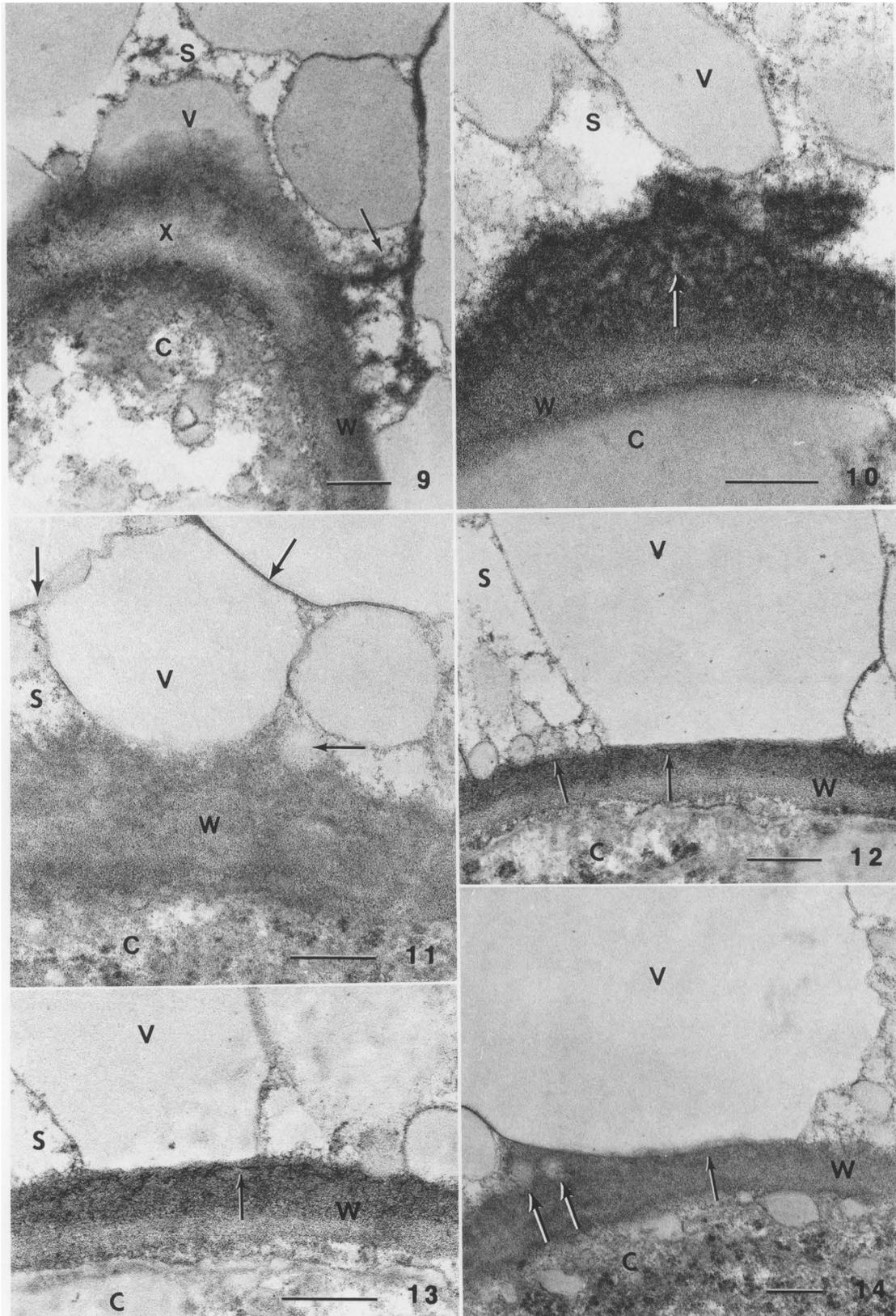
Wall matrix at the wall surface facing the secretory cavity was loosely aggregated, as contrasted to the wall region adjacent to the plasma membrane (Figs. 5, 7). At other positions along the wall surface the wall matrix entering the cavity contained particulate material that surrounded the vesicles as they were deposited in the cavity. Elsewhere along the wall facing the cavity, electron-dark bands of wall material were present at the wall-cavity interface, and wall matrix was associated with the surface of vesicles in the cavity (Fig. 5). As hyaline areas, such as the very elongated one, entered the cavity, all wall matrix and other hyaline areas between it and the cavity also were moved into the secretory cavity (Fig. 7).

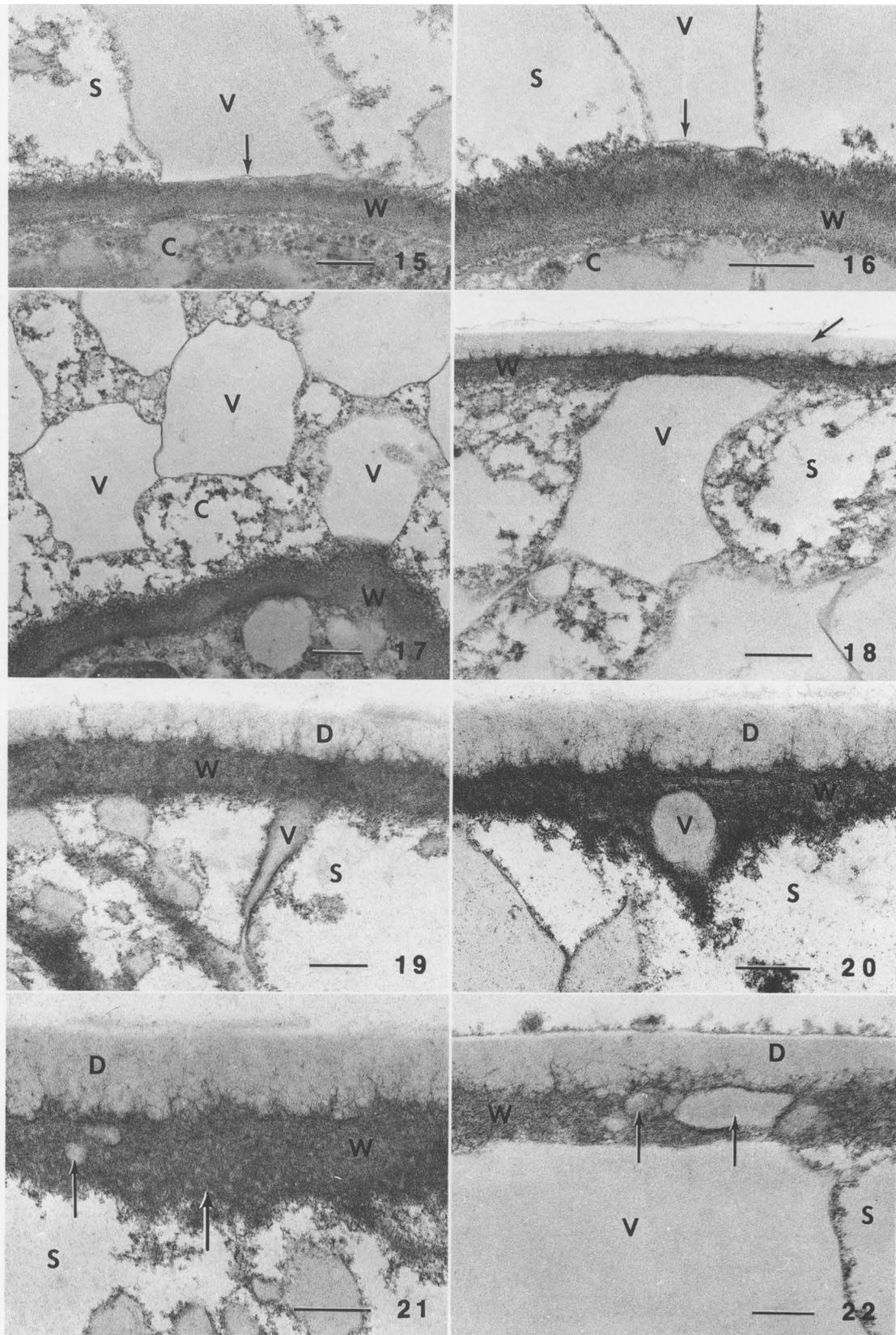
Formation of secretory vesicles in the enlarged secretory cavity—Secretory vesicle formation during this phase of gland morphogenesis was associated with electron-dense secretions formed in abundance in the disc cells (Kim and Mahlberg, unpublished data). These secretions, interpreted to be lipophilic in character, accumulated between the plasma membrane and the cell wall facing the secretory cavity (Fig. 8). Small and large accumulations were evident at this position outside the plasma membrane. Secretions moved directly through the cell wall to form a secretory vesicle in the cavity (Fig. 9). The wall region containing the secretion at the site of vesicle formation

Figs. 1–8. Secretory vesicle formation. 1. Loosening of wall (W) in outer region of this cell (C) wall initiates secretory cavity development (large arrow) immediately prior to vesicle formation. Cuticle (small arrow) on wall. Bar = 0.2 μm . 2. Somewhat enlarged secretory cavity (S) showing hyaline areas (arrows) of different sizes in wall (W) above disc cell (C). Bar = 0.2 μm . 3. Wall (W) of disc cell (C) showing possible origin of hyaline areas (small arrow) in wall near plasma membrane (large arrow). Other hyaline areas are evident throughout wall. Vesicle (V) is present in secretory cavity (S). Bar = 0.2 μm . 4. Plasma membrane-derived vesicle (small arrow) between this membrane and cell wall (W). Hyaline areas (large arrow) in wall and near wall surface facing secretory cavity (S). Loose wall matrix extends into secretory cavity. M, mitochondrion; secretion product (above C) in disc cell (C). Bar = 0.2 μm . 5. Several hyaline areas (small arrow) in tandem arrangement in wall (W) above disc cell (C). Membraned secretory vesicles (V) with electron-dense content in secretory cavity (S). Loose wall matrix with materials of different electron-density (as at large arrow) extends into secretory cavity. Bar = 0.2 μm . 6. Juncture region between two disc cells (C) showing electron-light hyaline areas (as at small arrow) in wall (W) in this region. Loose wall matrix (large arrow) and small vesicles (V) evident in secretory cavity (S). Bar = 0.2 μm . 7. Wall (W) of disc cell (C) showing hyaline area (arrow) extending from wall into secretory cavity (S). Other hyaline areas are in wall. Loose wall matrix extending into cavity from wall includes vesicles (V) with electron-dense content. Similar electron-dense content is evident in hyaline area at arrow. Bar = 0.2 μm . 8. Quantity of electron-dense secretory product (small arrow) shown between plasma membrane (large arrow) and disc cell wall (W). Bar = 0.4 μm .

Figs. 9–14. Vesicle formation in secretory cavity. 9. Wall (W) of disc cell (C) showing secretory product (X) as a light area in wall below a developing vesicle (V) in secretory cavity (S). Wall without secretory product (at W) is more dark in appearance. Secretory material is interpreted to be accumulating in vesicle (V). No bounding membrane is evident between wall and vesicle contents, whereas the vesicle is separated from contents of secretory cavity. Other vesicles are in secretory cavity. Loose wall matrix (arrow) extends into secretory cavity. Bar = 0.2 μm . 10. Wall (W) of disc cell (C) showing mottled appearance of wall facing secretory cavity (S) containing vesicles (V). Light mottles (arrow) are interpreted to be secretory product in wall. Loose wall matrix, at right, extends into cavity. Bar = 0.2 μm . 11. Wall (W) of secretory cell (C) showing enlarged vesicle (V) in cavity (S). Membrane is evident (large arrows) around this, and adjacent large vesicle, except at portion of vesicle in contact with wall. A small vesicle (small arrow) is evident embedded in wall. Bar = 0.2 μm . 12. Wall (W) of disc cell (C) showing presence of short segments of back bands (arrows) in the outer region of wall and adjacent to vesicle (V) with no membrane evident where vesicle contacts wall. Similar bands are evident in wall adjacent to several small vesicles partially embedded in wall (left). S, secretory cavity. Bar = 0.2 μm . 13. Wall (W) of disc cell (C) showing black bands (arrow) in wall and in close proximity to surface of vesicle (V) in contact with wall. S, secretory cavity. Bar = 0.2 μm . 14. Vesicle (V) showing presence of membrane (small arrow) along most of its surface where it contacts wall (W). Two small quantities of secretion material forming vesicles are evident in wall (large arrows). C, disc cell. Bar = 0.2 μm .







was lighter, as well as thicker, than where a vesicle was not being formed.

At other localized positions the wall appeared mottled from the presence of numerous small electron-light quantities of secretions distributed throughout the thickened wall (Fig. 10). The secretions that accumulated outside the plasma membrane composed the electron-light material in the wall.

New vesicles appeared as dome-shaped quantities of electron-dense secretions (Fig. 9). Vesicles with a broad surface of secretory product in contact with the wall were interpreted to be in the process of accumulating secretions. The vesicle increased in size from the accumulation of secretions passing through the wall (Fig. 11). The surface of the vesicle exposed in the cavity was bounded by a membrane, whereas none was evident where the secretion was in contact with the wall. Upon emerging from the wall it carried with it loose wall matrix that imparted an irregular dark appearance to the surface of the vesicle (Fig. 9).

Vesicle development was associated with formation of a membrane along the surface where the vesicle contacted the wall. Membrane formation was preceded by the appearance of electron-dark bands of material in the wall in the vicinity of vesicles (Figs. 12, 13). These bands of material aggregated under the secretory product in vesicles and contributed to formation of the vesicular membrane (Fig. 14). This new membrane became continuous with that portion of the membrane delimiting the vesicle surface in the cavity resulting in the formation of a continuous membrane around the enlarged vesicle.

Organization of the membrane at the vesicle-wall interface formed a smooth surface at this interface, whereas elsewhere, loosened wall matrix imparted an irregular appearance to the wall facing the cavity (Figs. 15, 16). The membrane became evident at the vesicle-wall interface (Fig. 15) and subsequently was observed as a distinctive membrane above the wall (Fig. 16). Enlarged vesicles at this stage in development occasionally were somewhat constricted where they contacted the wall.

The vesicle detached from the wall when continuity of the membrane in the wall was completed with that portion of the membrane surrounding the vesicle in the cavity (Fig. 17). The appearance of the vesicle at the left, with its flat and somewhat narrowed base, was indicative that it had detached from the wall. The similar appearance of the vesicle in the center of the figure also was suggestive

that it had detached from the wall, whereas the enlarged vesicle at the right in the figure may be approaching the stage of detachment from the cell wall.

Vesicles of small size also formed as secretions moved through the wall. Frequently, small quantities of secretions were detectable in the wall (Figs. 11, 14) and presumably entered the cavity as small membraned vesicles (Figs. 9, 12). Dark bands related to membrane material in the wall were evident in the proximity of the small vesicles (Fig. 12).

Wall matrix released into the cavity from the wall surface accumulated as dense material between the tightly packed vesicles (Fig. 9) or as loose aggregates of material throughout the secretory cavity when vesicles were not tightly packed in the cavity (Fig. 17). At surfaces where membranes of adjacent vesicles contacted each other in the cavity, the membranes typically became very dense, probably because of the trapped and compressed wall material between vesicles (Figs. 9, 11).

Vesicles released into the cavity distributed their secretory contents to the sheath surface (Fig. 18). Vesicles fused with the subcuticular wall (Fig. 19), whereupon their contents were deposited in this wall as large quantities of secretions (Fig. 20). At other positions the secretions were dispersed and imparted a mottled appearance to the wall which also could include larger quantities of secretions (Fig. 21).

Secretions contributed to thickening of the cuticle (Fig. 22). These new components for cuticle were added at the wall-cuticle interface where they imparted an irregular appearance to the cuticle. Although secretions in the subcuticular wall could be large in volume, only small quantities were detected in contact with the cuticle (Figs. 21, 22). Wall matrix surrounded the pockets of secretions as the secretions moved through the subcuticular wall to the cuticle. Typically, small quantities of secretions were deposited irregularly throughout the cuticle with some wall matrix remaining between these new deposits of cuticle and imparting a dendroid appearance to the cuticle (Figs. 21, 22).

DISCUSSION

Secretory activity of disc cells in relation to secretory cavity formation and development can be associated with two broad phases of gland development (Hammond and Mahlberg, 1978; Kim and Mahlberg, 1991; Mahlberg and

Figs. 15–22. Vesicle release from cell wall and association with sheath. **15.** Vesicle (V) in contact with wall (W) of disc cell (C) showing appearance of a membrane (arrow) at vesicle-wall interface. This interface is sharply demarcated, whereas in the secretory cavity (S), adjacent to vesicle, loose wall matrix is evident at wall surface and in the cavity. Bar = 0.2 μm . **16.** Vesicle (V) at wall (W) of disc cell (C) showing vesicle membrane (arrow) above wall surface. S, secretory cavity. Bar = 0.2 μm . **17.** Vesicle (V, right) shown associated with disc cell wall (W), whereas vesicle (V, left) is positioned a short distance above wall. The flat contour of the lower side of vesicle (V, center) is positioned a short distance above wall. The flat contour of lower vesicle surface (V, left and center) suggests that these vesicles were released into secretory cavity (C) from wall. Bar = 0.2 μm . **18.** Sheath showing vesicle (V) in contact with subcuticular wall (W) under cuticle (arrow). Electron-dense material is in secretory cavity (S). Bar = 0.2 μm . **19.** Vesicle (V) fused with subcuticular wall (W) under cuticle (D). Other vesicles (left) in secretory cavity (S) are in contact with wall. Bar = 0.2 μm . **20.** Secretion of vesicle (V) embedded in subcuticular wall (W) under cuticle (D). S, secretory cavity. Bar = 0.2 μm . **21.** Subcuticular wall (W) showing two large pockets of secretions from vesicles (small arrow) and mottled appearance of wall with numerous small pockets of secretions (area above large arrow) indicating that contents of some vesicles were dispersed into wall. D, cuticle; S, secretory cavity. Bar = 0.2 μm . **22.** Subcuticular wall (W) contains quantities of secretions of different sizes adjacent to and in contact with cuticle (D). Secretion material (arrows) appears to become incorporated into cuticle. S, secretory cavity; V, large vesicle below wall. Bar = 0.2 μm .

Kim, 1991). During the initial phase of cavity development the disc cells form extracellular hyaline areas that give rise to secretory vesicles in the secretory cavity. During the later phase of gland development, when plastids are associated with secretions passed outside the plasma membrane (Kim and Mahlberg, unpublished data), these secretions pass through the disc cell wall to become deposited in the secretory cavity.

Hyaline areas appear in the disc cell wall upon initiation of the secretory cavity and may be related in origin to membraned vesicles formed by the plasma membrane. The origin and morphology of similar vesicles outside the plasma membrane have been described for other cells (Mahlberg, Olsen, and Walkinshaw, 1971; Mahlberg et al., 1974). Although these authors reported the vesicles to give rise to secondary vacuoles in the cell, it is possible that such vesicles could remain outside the plasma membrane and contribute to the formation of hyaline areas.

Vesicle-like features in epithelial cell walls of latex ducts have been reported to be possibly associated with wall degradation during duct formation (Wittler and Mauseth, 1984), but it is unknown whether any relationship might exist between these features and the vesicles associated with the secretory process in glands of *Cannabis*.

Hyaline areas appear in the disc cell wall only after secretory cavity initiation, and subsequently become numerous throughout the wall during this phase of cavity development. They are interpreted to arise repeatedly along the wall surface adjacent to the plasma membrane, possibly from membraned vesicles that somehow pass into the wall. The observed progressive increase in size and tandem arrangement of hyaline areas toward the wall surface facing the secretory cavity support the interpretation that they move through the wall, and subsequently enter the secretory cavity. Upon entering the secretory cavity they give rise to secretory vesicles (Kim and Mahlberg, 1991).

Hyaline areas are detected only during the early phase of secretory cavity formation and contributed to the secretory vesicle complement during this phase of secretory cavity development. It is assumed that as hyaline areas distend to become secretory vesicles in the secretory cavity they become loaded with secretions. The origin of these secretions and the mechanism of secretion transfer to these vesicles during the early phase of secretory cavity development are not known.

During the later phase of secretory cavity development the secretions outside the plasma membrane pass directly through the disc cell wall. They pass through the wall as ultrastructurally conspicuous quantities of secretions in the wall. The vesicle emerging into the secretory cavity at the wall surface is covered with a membrane derived from the wall. This membrane typically possesses wall matrix components on its outer surface as it lifts off the wall during enlargement of the secretory vesicles. It increases in surface area as the vesicle enlarges from accumulated secretions passed through the wall. Precursors for membrane formation are probably derived from components in the wall.

While the developing vesicle is being loaded with secretions, no membrane is evident where the secretion contacts the wall surface. However, an enlarged vesicle is observed to possess a membrane at this surface when

it detaches from the wall to become a free vesicle in the secretory cavity (Mahlberg and Kim, 1991). The vesicle surface in contact with the wall derives its membrane from dense bands of material that become evident in the wall. These bands are first detected as short dense fragments at different positions throughout the wall. The dark bands are interpreted to move through the wall to the immediate proximity of the vesicle. The bands appear to become linearly arranged in the wall as a membrane forms across the somewhat narrowed base of the vesicle. Thereafter, the vesicle detaches from the wall to become a free secretory vesicle in the secretory cavity (Hammond and Mahlberg, 1978; Oliveira and Pais, 1990; Mahlberg and Kim, 1991). The secretory cavity contains numerous such vesicles of different sizes that are formed over a period of time during gland development.

Secretory vesicles contribute precursors for cuticle formation (Mahlberg and Kim, 1991). Upon contacting the subcuticular wall the vesicles distribute their contents into the wall where they appear as accumulations of different sizes. This directional flow of secretory materials from disc cells to sheath, rather than in the opposite direction, is supported by several observations: a large number of hyaline areas appear in the wall adjacent to the disc cells during early formation of the secretory cavity, whereas none are evident in the subcuticular wall; vesicles and other structural materials in the secretory cavity are most abundant at the disc cell wall surface, and less so in the subcuticular wall (Kim and Mahlberg, 1991); secretions in the cytoplasm of disc cells, external to the plasma membrane and in the cell wall are related to secretions from plastids (Kim and Mahlberg, unpublished data), whereas there is no source for formation of secretions in the subcuticular wall; secretions in the disc cells are similar in density to secretions external to the plasma membrane, in the disc cell wall and in vesicles in the cavity; and, both cuticle and subcuticular wall of the sheath become progressively thicker with the presence of vesicle contents in the secretory cavity during gland development indicating a flow of precursors toward the sheath rather than from the sheath to the disc cells (Kim and Mahlberg, 1991; Mahlberg and Kim, 1991).

The continued production of lipophilic secretory vesicles at the plasma membrane-wall interface relates to the composition and role of vesicle contents. Vesicles probably contain fatty acids associated with cuticle formation (Kolattukudy, 1980) which thicken the cuticle of the developing gland (Mahlberg and Kim, 1991). There also are present in the gland volatile monoterpenes and sesquiterpenes (Malingré et al., 1975; Croteau and Johnson, 1984; Hammond, Kim, and Mahlberg, unpublished data) that contribute to plant odor (Hood, Dames, and Barry, 1973) as they volatilize from the cuticular surface. We speculate that their evaporation from the gland surface, representing continued loss of monoterpenes from the secretory cavity, may contribute to the continued production of additional secretory vesicles at the disc cell wall surface.

The formation and apparent dynamic movement of membraned secretory vesicles in the secretory cavity is a unique phenomenon for disc cells, but may have application to cells in general. Its occurrence is made observable because of the enlarged secretory cavity which shows, in

part, an exploded view of the cell wall. It also provides insight into the mechanism for secretion of cuticular and waxy components in dermal and other cells. This study supports the interpretation that such lipophilic components are secreted directly into and through the wall from the plasma membrane surface.

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