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Stanley R. Gawlik

St. John Fisher University

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Abstract

A study of developing transmitting tissue of *Lilium Leucanthum* pistils was undertaken in order to correlate structure with function. Lining the stylar canal are stigmatoid cells which contain a secretory zone consisting of a labyrinth of wall ingrowths characteristic of transfer cells. The functional feature of the labyrinth is a high surface-to-volume ratio that facilitates an intensive transmembrane flux of solutes. Stigmatoid cells in various stages of development and maturation have been investigated with the aid of electron and light optics in conjunction with cytochemical techniques. During the development of the secretory zone, vesicles, formed by hypersecretory dictyosomes, fuse with the plasma membrane and contribute their contents to the growing wall. The pattern of secretory zone development is basipetal and is associated with initiation of chemotropism. In a mature pistil large crystals, having a basipetal pattern of development, and sensitive to protease, can be observed in the cytoplasm of stigmatoid cells. At anthesis, degradation of the crystal can be observed in the cells of the stigma surface and progresses basipetally as the pistil ages. The role of the crystal is uncertain. Immature pistils cultured in the presence of labeled proline take up the label which at maturity of the pistil is transferred to the canal of the pistil. The label is found in the crystals and secretory zone of the stigmatoid cells. Pollen tubes growing in the canal of a labeled pistil take up the label.

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AN ULTRASTRUCTURAL STUDY OF TRANSMITTING TISSUE DEVELOPMENT IN THE PISTIL OF *LILIUM LEUCANTHUM*¹

STANLEY R. GAWLIK

Department of Biology, St. John Fisher College, Rochester, New York 14618

ABSTRACT

A study of developing transmitting tissue of *Lilium leucanthum* pistils was undertaken in order to correlate structure with function. Lining the stylar canal are stigmatoid cells which contain a secretory zone consisting of a labyrinth of wall ingrowths characteristic of transfer cells. The functional feature of the labyrinth is a high surface-to-volume ratio that facilitates an intensive transmembrane flux of solutes. Stigmatoid cells in various stages of development and maturation have been investigated with the aid of electron and light optics in conjunction with cytochemical techniques. During development of the secretory zone, vesicles, formed by hypersecretory dictyosomes, fuse with the plasma membrane and contribute their contents to the growing wall. The pattern of secretory zone development is basipetal and is associated with initiation of chemotropism. In a mature pistil large crystals, having a basipetal pattern of development, and sensitive to protease, can be observed in the cytoplasm of stigmatoid cells. At anthesis, degradation of the crystal can be observed in the cells of the stigma surface and progresses basipetally as the pistil ages. The role of the crystal is uncertain. Immature pistils cultured in the presence of labeled proline take up the label which at maturity of the pistil is transferred to the canal of the pistil. The label is found in the crystals and the secretory zone of the stigmatoid cells. Pollen tubes growing in the canal of a labeled pistil take up the label.

THE PATHWAY of the pollen tube in angiosperms has been described or reviewed by Iwanami (1959), Esau (1965), Rosen (1968), Linskens and Kroh (1970), and Tilton and Horner (1980). In most angiosperms, such as petunia (Konar and Linskens, 1966) and cotton (Jensen and Fisher, 1969), the styles of the pistil are solid. The growing pollen tubes penetrate the central core of the style and grow intercellularly through the transmitting tissue which is cytologically different from surrounding tissue.

A second type of style (mostly found in monocots) has a hollow core which provides a canal for the growing pollen tubes (Iwanami, 1959; Vasil, 1974; Clarke et al., 1977). *Lilium* possesses a single layer of stigmatoid cells which surrounds the canal. These cells are cytologically distinct from the surrounding parenchyma cells. They are smaller, more densely cytoplasmic with large nuclei, and where they abut the canal, possess thicker convex cell walls. These cells, lined up end to end in vertical rows, form a multitude of narrow, regular grooves which run from the ovary to the stigma. Crang (1969) studied the fine structure of stigmatoid and parenchyma cells of *Lilium philippense* styles after pollination. Electron micrographs

of stylar cells, at varying intervals of time following pollination, revealed destruction of stylar parenchyma cells which could be related to pollen tube passage. The canal (stigmatoid) cells, following passage of the pollen tubes through the canal, remained intact for at least four days following pollination. Rosen and Thomas (1970) described the structure and function of secretory cells of lily pistils. The cells which line the canal throughout its length are different than the cells of the stigmatic surface. As a result, they referred to these as canal cells rather than stigmatoid cells. The canal cells were described as having a domed surface on the side facing the canal. The cell wall at the domed surface is smooth on the exterior and highly convoluted on the interior of the cell with a maximum thickness of 14 μ m. They referred to this area as the secretion zone of the cell because of its similarity to the cell walls of transfer cells described by Gunning and Pate (1969). The cells on the stigmatic surface lack the elaborations characteristic of the secretion zone.

Rosen (1962) defined chemotropism "as movement by growth toward or away from an external substance or substances." For many years it has been observed that the pistil influences the direction of growth of pollen tubes. The subject of chemotropism has been reported on and extensively reviewed by Rosen

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(1961, 1962, 1968), Linskens (1964), and Linskens and Kroh (1970). Rosen (1961) reported that the exudate of the stigma surface stimulated both germination and pollen tube growth of *Lilium longiflorum* pollen. Welk, Millington and Rosen (1965), working with *Lilium leucanthum* and *L. regale*, observed that pistil tissue which contains stigmatoid (canal) cells possesses chemotropic activity which progresses basipetally with age. The activity was present in the stigma 3–5 days prior to anthesis. Yamada (1965) cytochemically analyzed the mucilaginous material found in the stylar canal of lily following pollination, and suggested that the substance was a source of nutrition for the growing pollen tube. Rosen and Gawlik (1966) studied the ultrastructure of pollen tubes growing in compatible and incompatible styles of *L. longiflorum*. The growing zone at the tip of the pollen tube isolated from a compatible style is characterized by deep embayments in the wall. These embayments were not present in younger tubes near the stigma surface, nor in tubes grown in incompatible styles. It was suggested that the pollen tube possesses a heterotrophic type of nutrition, first utilizing material stored in the grain until depleted, and then, if compatible, taking in the material secreted by stigmatoid (canal) cells via the embayments. Heslop-Harrison (1975) reported that in *Liliceae* the products of the cells lining the stylar canal are responsible for the self-incompatibility reaction.

The secretions of the stigmatoid tissue found on the stigma surface and in the stylar canal have been implicated in chemotropism, nutrition, and compatibility. No reports have been found in the literature which describe the fine structure of the stigmatoid cells of *Lilium* during their development. Such a study may relate cellular structure with the change in function.

MATERIALS AND METHODS—Pistils utilized throughout this study were from *Lilium leucanthum* var. *centifolium*, Black Dragon, obtained from the Oregon Bulb Farms, Sandy, Oregon. The plants were grown in the greenhouse under natural light conditions. Pistils of different ages, prior to anthesis and after anthesis, were immersed in fixative and subdivided into five parts: stigma lobes, sub-stigma, mid-style, lower style, and ovary. Tissue was fixed at room temperature in a formaldehyde-glutaraldehyde fixative buffered to pH 7.2 with cacodylate according to Karnovsky (1965). Tissue was then rinsed in buffer and apportioned into two identical batches, half for electron microscopy, and half for light microscopy. Tissue to be used for fine structure analysis was

postfixed in cold 1% OsO_4 buffered as for fixation. Dehydration was accomplished through an ethanol series and propylene oxide, and embedded in Epon according to Luft (1961). Sections were placed on copper grids, stained with uranyl acetate and lead citrate, and examined. For light microscopy the tissue was embedded in glycol methacrylate according to Feder and O'Brien (1968). Half-micrometer sections were cut and placed on clean glass slides. Sections were then subjected to cytochemical assays for nucleic acids, polysaccharides, and proteins. For identification of DNA and polysaccharides, the Feulgen and PAS reactions according to Feder and O'Brien (1968) were employed. To identify proteins, acid fast green according to Jensen (1962) and the acrolein-Schiff reaction according to Van Duijn (1961) were employed. For identification of RNA, azure B according to Jensen (1962) was used.

Enzymatic digestion of aldehyde fixed tissue, prior to or after embedding in glycol methacrylate, was used to identify selected cell components. Incubation in 1% pronase at pH 7.5 for 5 hr at 37 C was used to digest proteins. Incubation in 1% ribonuclease ($5 \times$ crystalline; bovine pancreas) at pH 7.5 for 5 hr at 37 C was used to digest RNA. For DNA digestion, 1% deoxyribonuclease (bovine pancreas) at pH 7.5 with 0.001 M MgSO_4 for 5 hr at 37 C was employed. Controls were incubated in distilled water under the same conditions and in the enzyme after autoclaving for 1 hr at 126 C.

For studies of growth in vitro and autoradiography, pistils were cultured in test tubes containing Nitsch's medium (1951) and 50 μg of gibberellic acid (80%) according to Been (1966). Buds of varying size were detached from the plant, and surface sterilized with 95% ethanol. Sepals, petals, and stamens were removed, and, with a 5-mm pedicel still attached, the pistils were placed in culture tubes containing 2 cc of media, with or without 5 μCi $\text{L}-(5\text{-}^3\text{H})$ -proline. In studies utilizing autoradiography, pistils were transferred to cold medium for 24 hr when style elongation was complete. Half the pistils were then pollinated and allowed to stand 1 day prior to harvesting. Harvesting was accomplished by placing the pistils into fixative, slitting them longitudinally, removing the pollen tubes from styles of pollinated pistils, and subdividing selected areas of the pistils. Sectioned glycol methacrylate embedded material was placed on slides and coated with Ilford L-4 liquid emulsion according to Caro and van Tubergen (1962). Slides were incubated for 6 days and developed in Kodak D-19.

RESULTS—In *Lilium leucanthum*, var. *centifolium*, the minimum bud length observed prior to anthesis was 15 cm and the maximum 17 cm. The pistil length was 16.5 cm from the top of the stigma to the base of the ovary in maximum size buds. The ovary measured 5 cm in length. In such mature pistils the stigmatoid cells lining the canal are domed on the side facing the canal with a width of 20–25 μm , a height of 35–40 μm , and a length of 45–50 μm . The domed region of the stigmatoid cell possesses a wall labyrinth with extensions that project into the cytoplasm (Fig. 1, 2). This cell wall will be referred to as the secretion zone (SZ) of the cell (Rosen and Thomas, 1970). The basal portion of the cell abuts parenchyma tissue. The SZ is permeated with extensions of the cytoplasm. Occasionally mitochondria, lipid droplets, plastids, and ribosomes can be seen in these extensions (Fig. 3). The SZ stains heavily in the PAS reaction as do starch grains and cell walls of the parenchyma (Fig. 4).

Young stigmatoid cells sampled from a 4-cm bud can be seen in Fig. 5 and 6. The cells are roughly cuboidal, ranging between 15 and 17 μm . The cell wall facing the canal is flat and thin with no evidence of a labyrinth or projection. A large nucleus is surrounded by a dense cytoplasm. In comparison to mature stigmatoid cells, there is less rough endoplasmic reticulum, fewer vesicles and a complete absence of starch.

In mature pistils the surface of the stigma is covered with papillae of varying sizes. Some papillae contain up to four cells with large nuclei and a dense cytoplasm. Figure 7 is a longitudinal section through such a papilla on the stigma surface. Residual PAS-positive secretion products can be observed adhering to the surface of the papilla. Cell wall elaborations can be seen in the terminal cell of the papilla. Electron micrographs of the terminal cell of a papilla (Fig. 8) show cell wall thickenings al-

though not as elaborate as the cell wall thickenings of stigmatoid cells lining the canal of the style. The same pattern of cell wall thickening was observed in the stalk cells of the papilla and in cells from the stigma surface. The cytoplasm of these former cells is characterized by vesicles of various sizes containing one to several osmiophilic droplets which are not observed in stigmatoid cells of the style. The secretion product of these cells, which stains with acid fast green, toluidine blue, and in the PAS reaction, can be seen adhering to the cell surfaces (Fig. 7, 8).

The first sign of development of the SZ in stigmatoid cells can be observed at the stigma surface of 12-cm buds. Pistils taken from 14.5 cm buds already have well developed stigmatoid cells on the stigma surface and in the sub-stigma region. Stigmatoid cells in the upper half of the style are intermediate in development while those in the lower half have not developed a SZ (Fig. 9) but do have the convex cell surface characteristic of mature stigmatoid cells. In stigmatoid cells which do have a convex cell surface and no SZ, vesicles can be seen fusing with the plasma membrane and presumably emptying their contents at the interface of the plasma membrane and cell wall (Fig. 10). The vesicles are 650 Å in diameter, have a fuzzy appearance and appear to originate from the dictyosome. In more mature stigmatoid cells, prior to anthesis, this type of activity was less frequent.

Frequently found in the cytoplasm of mature stigmatoid and parenchyma cells of the stigma, style and ovary are crystals not bounded by a membrane, which can reach a size of 16 to 20 μm (Fig. 11). These crystals were not found in the sepals, petals, stamens, leaves, stem, bulb, or roots, but only in the pistil. The crystals are PAS negative, Feulgen negative, and do not stain with azure B. They do stain when exposed to acid fast green or the acrolein-Schiff reac-

tion. Enzymatic digestion with DNase or RNase did not affect the crystals, however, they were removed with pronase. Fine structure observations of crystals reveal 130-Å subunits arranged in rows 350 Å wide (Fig. 12).

The first appearance of crystals in the developing pistil was in parenchyma cells of the stigma lobe taken from a 3-cm bud. At this stage in development the smallest crystals observed have a diameter of 0.3 μm and are membrane-bound. Smaller membrane-bound crystals were never found. The membrane-bound crystals next appear in the parenchyma of the sub-stigma region taken from a 4-cm bud. Presence of crystals in the parenchyma of the style was first observed in a pistil from a 5-cm bud. The largest membrane-bound crystal observed in developing pistils was 2.1 μm at its widest point (Fig. 13). Larger membrane-bound crystals were not observed. In occasional sections, a continuity between the membrane of the endoplasmic reticulum and the membrane surrounding the crystal can be observed (Fig. 13).

Membrane-bound crystals appear in the stigmatoid cells just prior to development of the SZ. When the pistils reach maturity, just prior to anthesis, only crystals lacking membrane are observed. Within 24 hr after anthesis the crystals found in the stigma lobe and sub-stigma region reveal signs of degradation (Fig. 14). Examination of pistils at intervals up to 10 days after anthesis revealed a basipetal pattern of degradation. Ten days after anthesis the population and size of the crystals were dramatically decreased in the stigma and sub-stigma region.

In vitro culturing of pistils was accomplished according to the method of Been (1966). Nine pistils isolated from buds ranging in size from 6.3 cm to 13.2 cm were allowed to grow for 10 days and then harvested. Examination of sections with a light microscope revealed the presence of mature stigmatoid tissue and protein crystals. For autoradiographic studies, pistils from 8- to 9-cm buds were cultured in the presence of 5 μC L-(5- ^3H)-proline. Autoradiographs of sectioned pollen tubes removed from pistils cultured in the presence of labelled proline revealed heavy silver grain deposition over the pollen tubes (Fig. 15). A section from the

sub-stigma region of a nonpollinated labeled pistil revealed silver grain deposition over the SZ of the stigmatoid cells and over the protein crystal (Fig. 16). Whether more label was in the protein crystal than in the surrounding cytoplasm could not be resolved using light microscopy.

DISCUSSION—Labyrinthine plant cell walls may be found in a variety of plant tissues. Gunning, Pate and Briarty (1968) employed the term "transfer cells" to describe those plant cells which possess a labyrinth of wall ingrowths. Gunning and Pate (1969) ascribe to transfer cells a role in the transport of solutes either into or out of a cell. The functional feature of plant cells possessing ingrowths of wall material is a high surface-to-volume ratio which facilitates an intensive trans-membrane flux of solutes. The fine structure of the labyrinthine walls of transfer cells bears a striking resemblance to the secretory zone (SZ) of stylar canal cells of *L. longiflorum* described by Rosen and Thomas (1970). The resemblance is so great that they refer to the canal cell as another type of transfer cell. In *L. leucanthum* both the stigmatoid cells which line the canal and those found on the surface of the stigma possess cell wall ingrowths similar to those of transfer cells.

Rosen and Thomas (1970) examined the fine structure of the secretory cells from the pistils of *L. longiflorum* and reported that the cells lining the canal are distinctly different from those on the stigma surface. The cells on the stigma surface lack the cell wall ingrowths which are characteristic of the cells lining the canal and transfer cells. On the basis of this difference Rosen and Thomas suggested that the term "canal cell" be used for the cells lining the canal, rather than "stigmatoid cells" which had been previously used (Esau, 1965). In *L. leucanthum*, however, the distinction based on the aforementioned morphological dissimilarity cannot be made. The cells on the stigma surface possess cell wall ingrowths, although they are not as elaborate as the ingrowths found in the cells lining the canal. Some cytoplasmic difference, however, is evident between cells on the stigma surface and those lining the canal. The cells on the stigma surface possess vesicles of varying size containing one to several os-

Fig. 13-16. 13. Membrane-bound crystal MCR. Continuity between endoplasmic reticulum and the membrane surrounding the crystal (arrow) can be seen. $\times 45,900$. 14. A degrading crystal from a stigma lobe harvested after anthesis. $\times 23,600$. 15. Autoradiograph of sectioned, unstained pollen tubes removed from pistils cultured in labeled proline. $\times 600$. 16. Unstained section from the sub-stigma region of a non-pollinated pistil fed labeled proline. $\times 800$.

miophilic droplets. These organelles are not found in the cytoplasm of the cells lining the canal. This suggests that the secretion product of the cells lining the canal and those on the stigma surface may differ. Since both are involved with secretion and may differ only in products secreted, it is suggested that the term "stigmatoid cell" be retained. The more extensive cell wall ingrowths of cells lining the canal increase the secretory area of the protoplast. The greater surface-to-volume ratio with the elaborated wall infoldings would facilitate trans-membrane flux. In these cells the surface area abutting the canal into which secretory material is deposited is less than that of the papillose cells which protrude and expose greater surface area.

Development of the SZ in stigmatoid cells progresses basipetally in the pistil. In a pistil taken from a 14-cm bud, it was noted that the stigmatoid cells of the stigma and sub-stigma region had developed SZ while mid-style was intermediate in development and the lower style had no SZ at all. In stigmatoid cells of the lower style from such a pistil, vesicles of dictyosome origin were observed fusing with the plasma membrane where the cell abuts the canal. Presumably the vesicles are contributing their contents to the developing labyrinth. Gunning and Pate (1969) reported that during the development of wall ingrowths in transfer cells intensive dictyosome activity can be observed. Coated vesicles, presumably of dictyosome origin, were seen near the dictyosomes and the plasma membrane. Gunning and Pate observed that the secretory cells of extrafloral nectaries of *Vicia faba* L. do not form ingrowths until sugar secretion starts, and suggest that it may "prove to be the rule that the specialization of transfer cell wall is synchronized with the onset of intensive transport." That development of wall ingrowths in the stigmatoid cells of *L. leucanthum* is also associated with initiation of secretion can be supported by two kinds of evidence. The development of the stigmatoid cell SZ progresses basipetally. The first sign of SZ development was observed at the stigma surface of a pistil from a 12-cm bud. In a pistil from a 14-cm bud the mid-style was intermediate in development and lower style had no SZ. This correlates with information on the initiation of chemotropic activity. Welk et al. (1965) sampled pistil segments of *L. leucanthum* at varying intervals prior to and after anthesis and assayed the segments for the presence of chemotropic activity. Segments from mature pistils that contained stigmatoid cells possessed chemotropic activity. Assays for chemotropic activity in young pis-

tils prior to anthesis showed that the activity progressed basipetally, commencing in the stigma of a 13-cm bud, appearing in the mid-style of a 14-cm bud, and finally present in the ovules 1-2 days after anthesis. On the basis of these two lines of evidence and observation of Rosen and Thomas (1970), it seems evident that stigmatoid cells are indeed another type of transfer cell.

The secretion products of plant cells are often attributed to the activity of hypersecretory dictyosomes, which produce vesicles that transport the secretions to the cell wall. Morr, Jones and Mollenhauer (1967) reported that the polysaccharide secreted by the outer root cap cells of *Zea mays* is mediated by the dictyosome. The cisternae of the dictyosome form large vesicles which fuse with the plasma membrane and discharge their contents, which then move through the cell wall and appear as droplets of hydrated polysaccharide adhering to the root tip. This type of activity has not been observed in mature stigmatoid cells after anthesis when secretory activity is occurring. Some other mechanism that transports secretion products to the canal and stigma surface must be sought.

A potential candidate for the precursor of the secretion product of the stigmatoid cells is the protein crystal found in parenchyma and stigmatoid cells of the pistil. As previously pointed out, the membrane-bound crystals first appear in the parenchyma cells at the distal end of the pistil and follow a basipetal direction of development. In stigmatoid cells membrane-bound crystals first appear after development of the SZ is initiated, and chemotropic activity is present. After anthesis the crystals lack a membrane and follow a basipetal pattern of degradation, first signs of degradation being at the stigma surface. Function of the protein is uncertain.

Pistils cultured in medium containing labeled proline show an accumulation of label in the SZ, and in the crystals. However, the resolution of the methods employed is not sufficient to determine whether more label is over the crystal or surrounding cytoplasm. Pollen tubes isolated from the canal of a proline-labeled pistil contain label, indicating that the label was in the secretory products transferred to the canal and that the growing pollen tubes were able to take up the label. Similar results were obtained by Dashek and Rosen (1966) who exposed lily pollen germinating in vitro to H³-proline. High resolution radioautographs of proline-labeled pollen tubes revealed a distribution of silver grains over tip cytoplasm and wall components. Proline can be utilized by growing pollen tubes in several ways:

incorporation into collagen-like nonmetabolizing proteins; intensify synthesis of proteins and amino acids; used as a source of nitrogen for synthesis of other amino acids and participate as a hydrogen acceptor in certain oxidation-reduction reactions (Britikov et al., 1964).

The secretory products of the pistil might be implicated in chemotropism, nutrition and/or incompatibility. Of these the most likely role of the crystal is that of nutrition since degradation of the crystal does not occur until anthesis, while chemotropism can be demonstrated prior to anthesis. It is not possible to speculate whether or not the crystals are directly involved in incompatibility, the mechanism of which is currently under investigation (de Nettancourt, 1977; Heslop-Harrison, 1982).

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