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Development and Ultrastructure of Glandular Trichomes in *Pelargonium* x *fragrans* 'Mabel Grey' (Geraniaceae)

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The glandular trichomes of leaves from *Pelargonium* x *fragrans* 'Mabel Grey' (Geraniaceae) were examined by light, scanning, and transmission electron microscopy. These trichomes had unicellular globular heads and stalks of different lengths and features. Two types were classified: Type I, with an elongated, large head and a short (100 μm), cylindrical stalk that was more apparent on the adaxial surface; and Type II, with a spherical, small head and a long (300 μm), conical stalk that was more pronounced on the abaxial surface. The ultrastructure of secretory cells from both types was distinguished by a well-developed endoplasmic reticulum, mitochondria, plastids, dictyosomes, and numerous vacuoles that likely were involved in the storage and transport of lipophilic substances. Plasmodesmata were frequent on the walls of the secretory and stalked cells. Here, we discuss the implication of structural differentiation in these trichomes.

Keywords: development, glandular trichomes, *Pelargonium*, secretion, ultrastructure

The metabolic process is often divided into the activities of excretion, secretion, and recretion (Frey-Wyssling, 1972). Accordingly, excretion is the means by which the secondary compounds produced by plant metabolism are eliminated, secretion is the removal of assimilation products without their entering the dissimilation metabolism, and recretion is the elimination of salts, thereby regulating the ion content within a cell.

In the secretory organs, substances are exuded either directly, to the outside, or indirectly, into the intercellular spaces. These substances may have considerable ecological importance, e.g., serving as agents of protection against animal attack. However, in contrast to the secretory substances found in animal cells, which generally consist of glycoprotein, those from plants usually are carbohydrates (Wagner, 1990).

Although natural essential oils have great commercial value, information is limited concerning the morphological, anatomical, and developmental mechanisms of the glandular trichomes responsible for their secretion (Bosabalidis and Tsekos, 1982; Bruni and Modenesi, 1983; Venkatachalam et al., 1984; Werker et al., 1985; Fahn, 1988; Isman, 2000; Skocibusic et al., 2006). These oils protect the aerial parts of a plant against herbivores and pathogens (Werker et al., 1993). Such properties also make the secondary metabolites in these secreted products desirable to the pesticide, pharmaceutical, flavoring, and fragrance industries (Duke and Paul, 1993; Hosokawa and Fukunaga, 1995).

Many studies have been performed on the chemical composition of essential oils. However, relatively little research has been devoted to defining the process of development and secretory mechanisms for oil-producing trichomes (Fahn, 1988). Lipophilic glandular trichomes show great variety in their morphologies, presumably because of different developmental processes and mechanisms. While there is considerable interest in the production of these valuable compounds (Werker, 1993; Wise and Croteau, 1999), aside from understanding the bioactivity and phytotoxicity of the glands that synthesize these oils, the developmental and structural biology of the secretory mechanism is not well known.

The chemical composition of essential oils has been widely studied, but the morphology and ultrastructure of secreting glandular trichomes have been examined in only a few species, including *Origanum* (Bosabalidis and Tsekos, 1982, 1984), *Thymus* (Brunni and Modenesi, 1983), *Salvia* (Venkatachalam et al., 1984), *Majorana* (Dudai et al., 1988), *Mentha* (Gershenzon et al., 2000; Turner et al., 2000), *Satureja* (Bosabalidis, 1990), *Hummulus* (Oliveira and Pais, 1990; Kim and Mahlberg, 2000), *Teucrium* (Antunes and Sevinate-Pinto, 1991), *Artemisia* (Duke and Paul, 1993; Stephen et al., 1993), *Nepeta* (Bourett et al., 1994), *Conium* (Corsi and Biasci, 1998), and *Cannabis* (Mahlberg and Kim, 1992, 2004).

The *Pelargonium* species are found primarily in South Africa, with a few other species occurring in tropical Africa, Australia, and a few islands in the Indian Ocean. Plants have now been introduced into Europe and Asia. Due to the importance of their oils, aromatic *Pelargonium* species have attracted attention from the perfume industry. However, most studies have focused on horticultural and agricultural aspects, rather than on the role of glandular trichomes in oil production.

Here, we have examined two aspects of *Pelargonium* trichomes: 1) the functional significance of their developmental and ultrastructural features, and 2) their sub-cuticular spaces in which these secretory substances accumulate.

MATERIALS AND METHODS

Plant Material

Plants of *Pelargonium* x *fragrans* 'Mabel Grey' were grown under greenhouse conditions at Konkuk University.

Light Microscopy

Hand-sections were cut by razor blade from freshly collected plant materials. The sections were fixed with FAA (formalin-acetic acid-ethyl alcohol) at 4°C for 12 h, then

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dehydrated in a graded ethanol series and embedded in paraffin. Blocks were cut using a rotary microtome, and the sections were hydrated through a decreasing alcohol series. They were then stained with safranin and fast green FCF, and observed under a light microscope (Axiphot II, Carl Zeiss, Germany).

Scanning Electron Microscopy

Specimens were fixed with 2% glutaraldehyde in 0.025 M phosphate buffer (pH 7.2) at RT for 2 h, then washed in the same buffer and post-fixed with 2% OsO_4 in distilled water at RT for 1 h. After washing in distilled water and dehydrating in ethanol, they were transferred to isoamyl acetate. The tissues were placed in a critical-point dryer (Bioradical E3000, Bio-Rad, USA), then mounted on aluminum specimen stubs, coated with 20 nm of gold-palladium in a sputter coater (JFC-1110E, JEOL, Japan), and photographed in a scanning electron microscope (JSM-840A, JEOL) at 20 kV.

Transmission Electron Microscopy

Leaf segments were fixed with a mixture of 2% glutaraldehyde in 0.025 M phosphate buffer (pH 7.2) for 2 h before being washed in the same buffer at RT for 5 min. Post-fixation was done for 2 h in 2% OsO_4 in distilled water, and the materials were rinsed for 5 min. Following dehydration in a graded ethanol series for 40 min, the samples were embedded in Spurr's resin. Semi-thin sections were stained with toluidine blue-basic fuchsin for approximately 30 s, then rinsed for 1 min in running water. Thin sections of silver to gold color were collected on copper grids, stained for 20 min with 1% uranyl acetate and for 10 min with lead citrate, and observed with a transmission electron microscope (JEM-2000 EX II, JEOL) at 80 kV.

RESULTS

Trichomes are quite common on *Pelargonium* leaves, stems, and flowers, with non-glandular and glandular bodies occurring on both the adaxial and abaxial surfaces of a leaf (Table 1). The non-glandular trichomes are uniseriate, pointed, and straight, and consist of thick-walled cells with numerous tiny spines. They are situated above the veins, on the leaf margins, and on the stems and petioles (Fig. 1). Glandular trichomes at various developmental stages are more numerous on the leaf abaxial surface (Fig. 1C, D) than on the adaxial surface (Fig. 1A, B). Leaves of *Pelargonium* have two types of capitate glandular trichomes. These are derived from protodermal cells by two anticlinal divisions in the perpendicular planes. The two types are easily distinguishable at all stages of development, with Type I showing an elongated, large head and a short, cylindrical stalk (Fig. 2A-D, 3A-D), while Type II has a spherical, small head and a long, conical stalk (Fig. 2E-H, 3E-H).

A mature Type I trichome consists of one head cell, three stalk cells, and one basal cell, compared with one head cell,

Table 1. Number of non-glandular and glandular trichomes on *Pelargonium* leaves per mm^2.

Leaf surface \ Trichomes	Non-glandular	Glandular	
		Type I	Type II
Adaxial	68	113	7
Abaxial	97	126	30

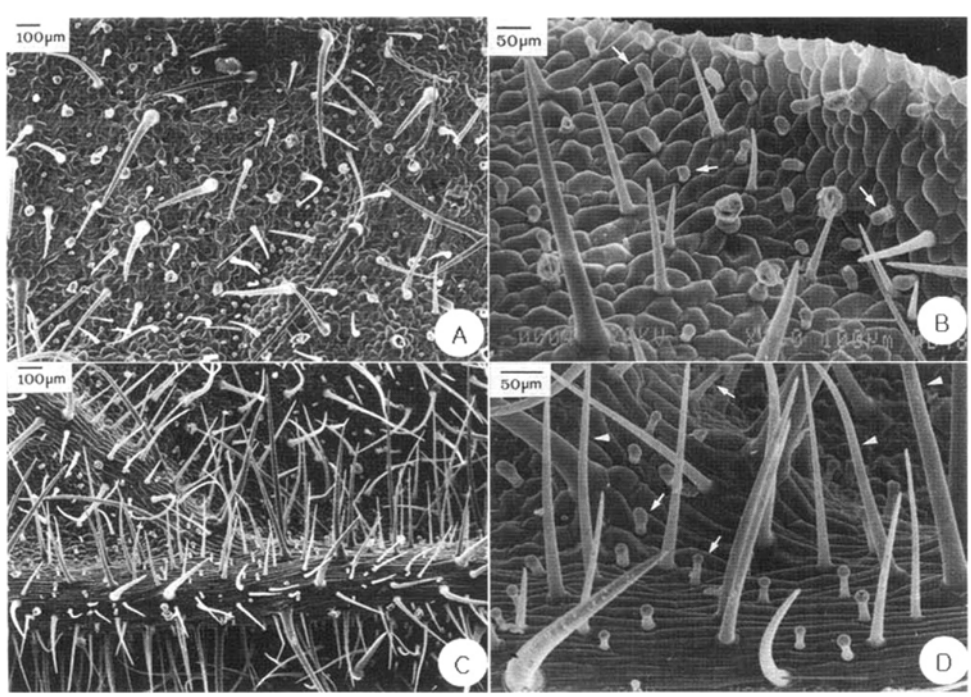

Figure 1. SEM of leaf surfaces showing non-glandular and glandular trichomes. (**A**) Adaxial leaf surface. (**B**) Close-up of Figure 1A. (**C**) Abaxial surface of leaf with dense mass of non-glandular trichomes on veins. (**D**) Close-up of Figure 1C.

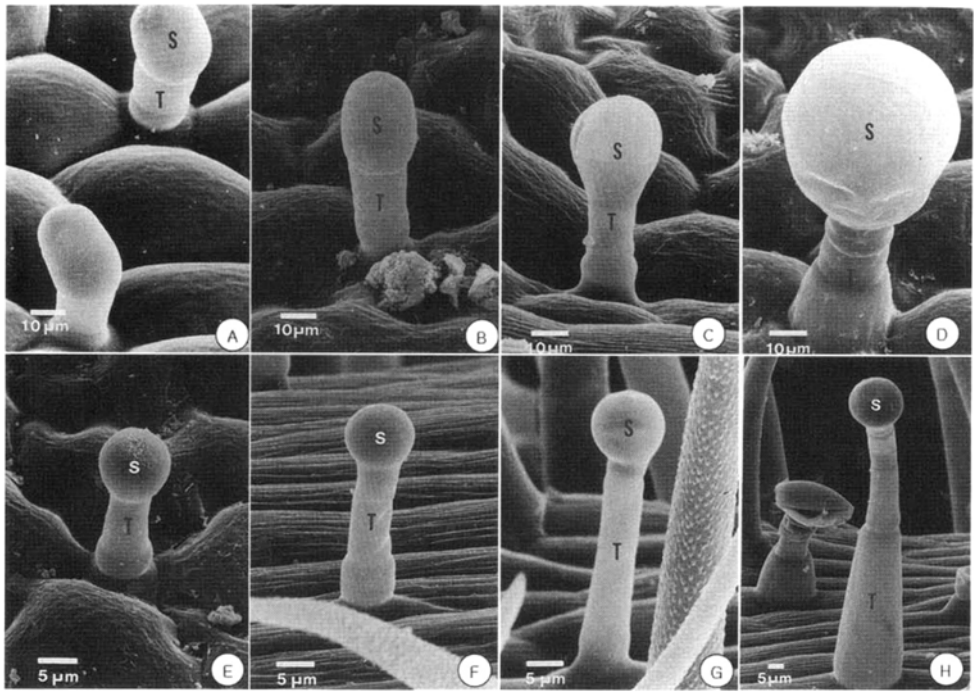

Figure 2. SEM for various stages of glandular trichome development. Short-stalked Type I (**2A-D**), and long-stalked Type II (**2E-H**). S, secretory cell; T, stalk cell.

Table 2. Number of cells consisting of glandular trichomes during trichome development.

Cell type \ Stage	Type I			Type II		
	I	II	III	I	II	III
Secretory	1	1	1	1	1	1
Stalk	2	2	3	2	4	5
Basal	1	1	1	1	1	1

Table 3. Average length and width of glandular trichomes.

Size (μm) \ Stage	Type I			Type II		
	I	II	III	I	II	III
Total length	47.1	63.4	92.2	57.3	181.1	287.1
Head width	17.5	16.3	28.8	8.8	22.5	32.5
Head height	18.8	23.8	35	10	27.5	42.5

five stalk cells, and one basal cell found for Type II (Table 2). The former can be about 100 μm long, with a head width of 30 μm (Fig. 2D, 3D; Table 3), while Type II trichomes may reach 300 μm long and 35 μm wide (Fig. 2H, 3H; Table 3). Both types originate from the protodermal cells by anticlinal divisions (Fig. 3).

The earliest observable stage of glandular trichome formation is the one-cell stage, in which a single epidermal cell enlarges and protrudes above the leaf surface. After considerable expansion above the leaf surface, this cell divides anticlinally (Fig. 4).

The two types of glandular trichomes have similar ultrastructures. Mitochondria, rough endoplasmic reticulum, plastids, ribosomes, and dictyosomes are dispersed throughout the cell (Fig. 5). In the secretory cells of mature glands, the mitochondria, rER, and vesicles derived from ER are the abundant cellular compartments. During the secretory phase, a large number of vacuoles in the upper cytoplasm commonly contain electron-dense substances that are accumulated in the secretory cavity (Fig. 5A, B). These secretory cells are often bowl-shaped, with a folded cuticle that assumes a dome-like form (Fig. 5C). The secretory cavity develops over the secretory cells, beginning in the outer cell wall by the loosening of the wall matrix. These materials are released from the secretory cell wall and accumulate in the secretory cavity (Fig. 5D). The initial loosening in the outer wall zone represents the development of distinctive hyaline areas between the fibrils that begin as small and elongated, often in a tandem arrangement (Fig. 5E, arrows). The hyaline areas also continue to be released from the wall in the cavity (Fig. 5F). A fully developed secretory cell has fewer cell organelles, but abundant secretory substances are usually common in the periplasmic and sub-cuticular spaces (Fig. 5G, 6A).

During maturation of the glandular trichome, the number and size of dictyosomal vesicles increase. In the fully active phase, secretory substances are accumulated in the sub-cuticular space that forms at the tip of each secretory cell. However, the cytoplasm in an older glandular trichome gradually degenerates, and the nucleus becomes vague (Fig. 6A). Secretory, basal, and stalk cells are frequently connected by many plasmodesmata (Fig. 6B, C). Stalk and basal cells have well-developed central vacuoles, and the cuticle of a glandular trichome thickens over time (Fig. 6B-E). The outer walls of the stalk cells are completely cutinized, forming a barrier against the free movement of substances between the glandular head and the leaf through apoplasts (Fig. 6C, E).

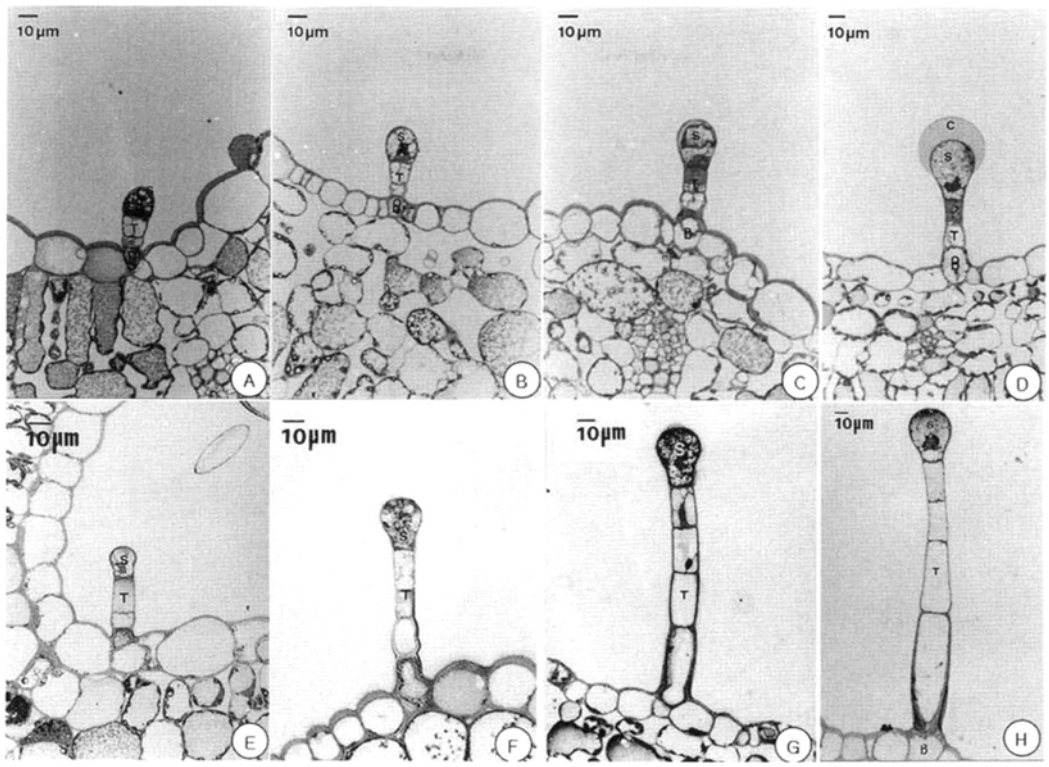

Figure 3. Light micrographs at various developmental stages of Type I (**3A-D**), and Type II trichome (**3E-H**). B, basal cell; C, secretory cavity; S, secretory cell; T, stalk cell.

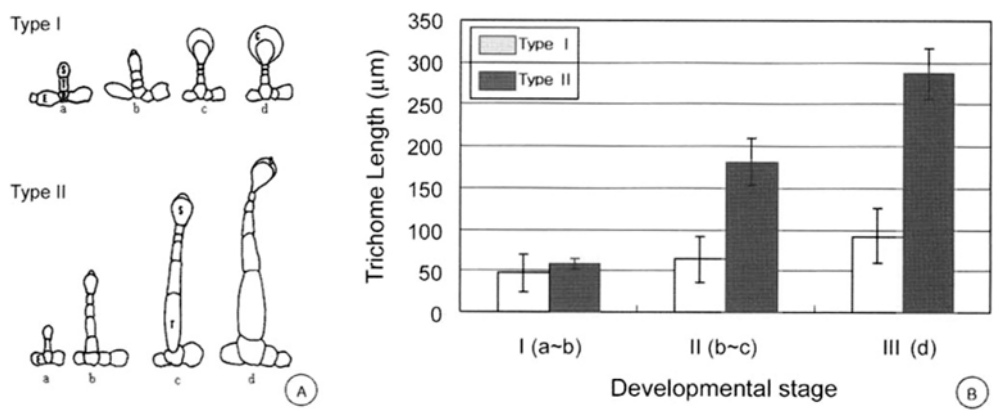

Figure 4. (**A**) Diagrammatic representation of glandular trichome development in *Pelargonium*. (**B**) Changes in total length of trichome. (**a-d**) Development of trichome. (**a**) Initiation occurs upon enlargement of protodermal cell that is bisected by periclinal division. (**b**) Stalk cell is separated by periclinal division. Secretory cavity formation starts in this stage. (**c**) Size of secretory cavity is extremely increased. (**d**) Mature glandular trichome consists of enlarged secretory cavity covered with sheath comprising cuticle and sub-cuticular wall. B, basal cells; C, secretory cavity; E, epidermal cells; T, stalk cells; S, secretory cells.

DISCUSSION

Our results demonstrate the development and ultrastructure of glandular trichomes in *Pelargonium* x *fragrans*. Although their two types differ in numbers of stalk cells, both have the same amounts of head and basal cells. Both glandular trichomes originate from the protodermal cells, and secretions accumulate in the sub-cuticular spaces. The secretory cells of glandular trichomes are involved in the release of diverse secondary products, including terpenoids and phenols (Werker et al., 1993; Ascensao et al., 1999; Hallahan, 2000; Turner et al., 2000).

A glandular trichome comprises a long, highly vacuolated stalk cell, a short sheath or barrier cell of smaller diameter, and a glandular head containing one cell. Although their superficial appearances differ, most of the ultrastructure of these two gland types is similar. During the pre-secretory phase, a glandular cell contains a large nucleus with a prominent nucleolus and a dense cytosol that is rich in ribosomes. Mitochondria, plastids, rER, and vacuoles are the most abundant organelles in secretory cells from mature glandular trichomes. This might reflect the high energy

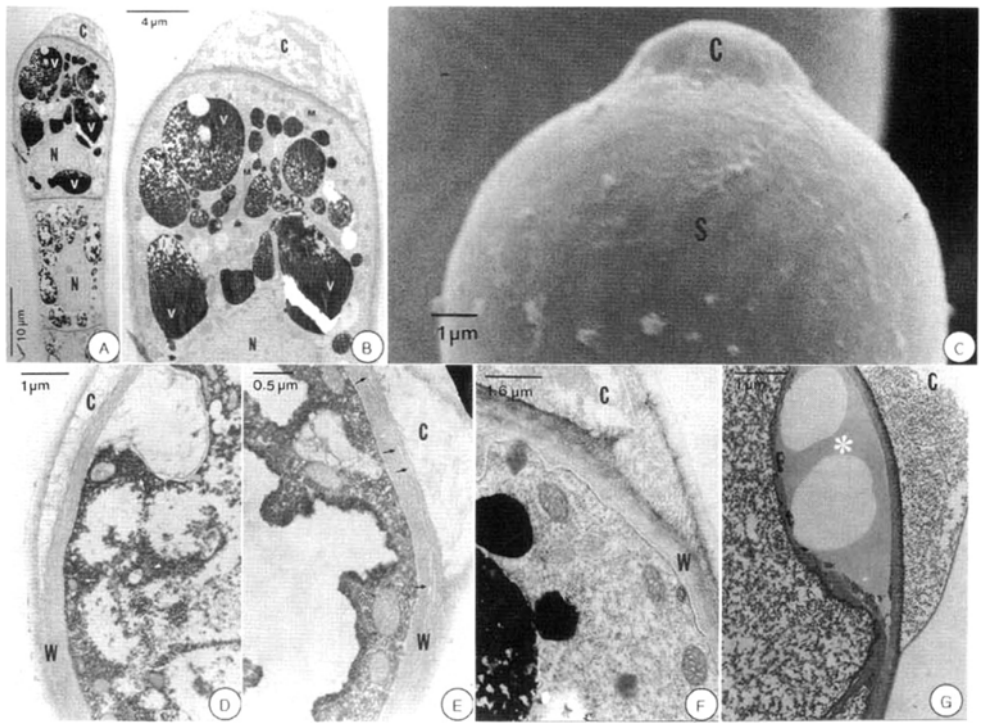

Figure 5. STEM of secretory cells in glandular trichomes. (**A**) Longitudinal section of Type II exhibiting head cell with cavity and elongated stalk cell containing prominent nucleus. (**B**) Higher magnification of head cell in Figure 5A. Note the osmiophilic material in vacuoles and the secretory substances accumulated within cavity. (**C**) Close-up of glandular head cell showing small cavity on central point. (**D**) Early formation of secretory cavity. (**E**) Elongated hyaline areas, in tandem arrangement, are evident in wall (arrows). (**F**) Fibrous materials on secretory cell wall appear loosely structured in secretory cavity. (**G**) Secretory substance accumulated in periplasmic space (asterisk). C, secretory cavity; N, nucleus; S, secretory cell; V, vacuole; W, cell wall.

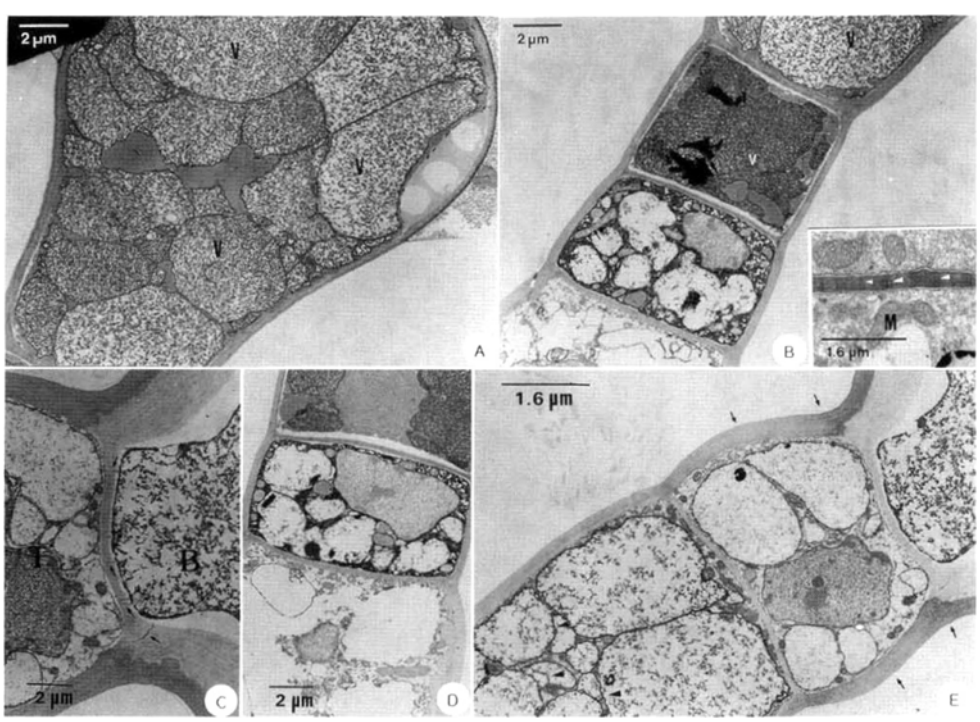

Figure 6. TEM micrographs of mature short-stalked trichome, Type I. (**A**) Secretory cell from developed glandular trichome showing numerous vacuoles and secretory substance. (**B**) Stalk cells of glandular trichome. Note longitudinal views of plasmodesmata on cross-wall of stalk cells (arrowheads, insert). (**C**) Enlarged portion of stalk and basal cells from glandular trichome. Note electron-dense cuticle on wall of stalk cell and thickened wall of basal cell. Plasmodesmata are also visible on cross-wall between these cells (arrows). (**D**) Stalk cells showing numerous vacuoles with electron-dense content. (**E**) Extended portion of Figure 6C showing difference in wall properties between stalk and basal cells (arrows). B, basal cell; M, mitochondrion; V, vacuole; T, stalk cell.

required for maintaining the extra-cellular location of secreted material against a concentration gradient.

Investigators have previously noted the involvement of plastids and sER in terpene secretion (Ascensao and Pais, 1998; Turner et al., 2000). However, the latter is absent from the secretory cells of *Pelargonium*-like *Origanum dictamnus* (Bosabalidis and Tsekos, 1982). This observation has led to a range of speculations concerning the biosynthesis site of secretory material. A lack of chloroplasts plus the presence of a barrier to apoplastic flow in the stalk indicates that the precursors for secretion components may originate from the mesophyll. Plasmodesmata in the periclinal wall of stalk cells may help transport photosynthates into the glandular cells, contributing to the regulation of the secretion rate.

The release of secretions from the lipophilic glands via cuticular rupture has been reported in *Newcastelia viscida* (Dell and McComb, 1978), *Cannabis sativa* (Hammond and Mahlberg, 1978), and *Thymus vulgaris* (Bruni and Modenesi, 1983). However, the trichomes of *Leonotis leonurus* evidence releases through the cuticular micropores (Ascensao and Pais, 1998). In all of these studies, the trichomes degenerate following that rupture. The stalk cells from *Pelargonium* x *fragrans* show cutinization of their lateral cell walls, and numerous plasmodesmata in the periclinal walls, but little other structural specialization, and a lack of chloroplasts. Similar characteristics are noted from the trichomes of Lamiaceae members (Bosabalidis and Tsekos, 1982; Fahn, 1988; Bourett et al., 1994).

During the final phase, secretory products accumulate between the cuticle and walls of the secretory cells. In *Pelargonium*, the bounding dermal sheath of the secretory cavity consists of a cuticle and a portion of the pectic outermost cell wall layer. This wall reinforcement along the cuticle may provide resistance to the secretory cavity, where large amounts of secretion are stored. An analogous mechanism for cavity formation has been suggested for the glandular trichomes of *Cannabis* (Kim and Mahlberg, 1991, 1995).

The presence of a cellulose reaction product in the secretory cell walls during progressive stages of secretory cavity development indicates its continuing role during cavity enlargement. Cellulose may function in the release of wall materials, e.g., the fibrils into the secretory cavity. These fibrils appear to be a precursor to the thickening of the subcuticular wall during enlargement of the cavity (Kim and Mahlberg, 1991, 2003). The wall loosens and secretions accumulate; as a result, a sub-cuticular space forms by the detachment of the cuticle and the outermost pectic layer of the cell wall. Accumulation of secretions in the sub-cuticular space gives a spherical shape to the trichome.

These accumulations are temporary in the sub-cuticular space, before the secretions are released when the cuticle ruptures. Afterward, the trichomes degenerate because of environmental damage to the leaf. The secretory material possibly is transported to the space between the plasma membrane and the cell wall, and subsequently accumulates in the secretory cavity. Cutinization of the side walls of stalk cells is frequently observed in glandular trichomes. It is generally assumed that these cutinized walls may block the back flow of secretions stored in the sub-cuticular space, thereby preventing the intoxication of mesophyll cells (Ascensao et al., 1997). Plasmodemata in the periclinal walls of stalk cells may enable the transport of photosynthates into the secretory cells, contributing to the regulation of secretion rates.

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LITERATURE CITED

Antunes T, Sevinate-Pinto I (1991) Glandular trichomes of *Teucrium scorodonia* L.: Morphology and histochemistry. Flora 185: 65-70

Ascensao L, Marques N, Pais MS (1997) Peltate glandular trichomes of *Leonotis leonurus* leaves: Ultrastructure and histochemical characterization of secretions. Intl J Plant Sci 158: 249-258

Ascensao L, Mota L, Caastro M (1999) Glandular trichomes on the leaves and flowers of *Plectranthus ornatus*: Morphology, distribution and histochemistry. Ann Bot 84: 437-447

Ascensao L, Pais MS (1998) The leaf capitate trichomes of *Leonotis leonurus*: Histochemistry, ultrastructure and secretion. Ann Bot 81: 263-271

Bosabalidis AM (1990) Glandular trichomes in *Satureja thymbra* leaves. Ann Bot 65: 71-78

Bosabalidis AM, Tsekos I (1982) Glandular scale development and essential oil secretion in *Origanum dictamnus* L. Planta 156: 496-504

Bosabalidis AM, Tsekos I (1984) Glandular hair formation in *Origanum* species. Ann Bot 53: 559-563

Bourett TM, Howard RJ, O'Keefe DP, Hallahan DL (1994) Gland development on leaf surfaces of *Nepeta racemosa*. Intl J Plant Sci 1155: 623-632

Bruni A, Modenesi P (1983) Development, oil storage and dehiscence of peltate trichomes in *Thymus vulgaris* L. Nor J Bot 3: 245-251

Corsi G, Biasci D (1998) Secretory structures and localization of alkaloids in *Conium maculatum* L. (Apiaceae). Ann Bot 81: 157-162

Dell B, McComb AJ (1978) Plant resins-their formation, secretion and possible functions. Adv Bot Res 6: 277-316

Dudai N, Werker E, Putievsky E, Ravid U, Palevich D, Halevy AH (1988) Glandular hairs and essential oil in the leaves and flowers of *Majorana syriaca*. Israel J Bot 37: 11-18

Duke SO, Paul RN (1993) Development and fine structure of the glandular trichomes of *Artemisia annua* L. Intl J Plant Sci 154: 107-118

Fahn A (1988) Secretory tissues in vascular plants. New Phytol 108: 229-257

Frey-Wyssling A (1972) Elimination processes in higher plants. Saussurea 3: 79-90

Gershenzon J, McConkey, Croteau R (2000) Regulation of monoterpene accumulation in leaves of peppermint (*Mentha* x *piperita* L.). Plant Physiol 122: 205-213

Hallahan DL (2000) Monoterpenoid biosynthesis in glandular trichomes of Labiate plants, *In* DL Hallahan, JC Gray, eds, Advances in Botanical Research, Vol 31. Academic Press, San

Diego, pp 77-120
Hammond CT, Mahlberg PG (1978) Ultrastructural development of glandular hairs of *Cannabis sativa* L. (Cannabaceae). Amer J Bot 65: 140-151
Hosokawa K, Fukunaga Y (1995) Production of essential oils by flowers of *Hyacinthus orientalis* L. regenerated *in vitro*. Plant Cell Rep 14: 575-579
Isman MB (2000) Plant essential oils for pest and disease management. Crop Protect 19: 603-608
Kim ES, Mahlberg PG (1991) Secretory cavity development in glandular trichomes of *Cannabis sativa* L. (Cannabaceae). Amer J Bot 78: 220-229
Kim ES, Mahlberg PG (1995) Glandular cuticle formation in *Cannabis sativa* L. (Cannabaceae). Amer J Bot 82: 1207-1214
Kim ES, Mahlberg PG (2000) Early development of secretory cavity of peltate glands of *Humulus lupulus* L. (Cannabaceae). Mol Cell 10: 487-492
Kim ES, Mahlberg PG (2003) Secretory vesicle formation in the secretory cavity of glandular trichomes of *Cannabis sativa* L. (Cannabaceae). Mol Cell 15: 387-395
Mahlberg PG, Kim ES (1992) Secretory vesicle formation in glandular trichomes of *Cannabis sativa* (Cannabaceae). Amer J Bot 79: 166-173
Mahlberg PG, Kim ES (2004) Accumulation of cannabinoids in glandular trichomes of *Cannabis* (Cannabaceae). J Indus Hemp 9: 15-36
Oliveira MM, Pais MS (1990) Glandular trichomes of *Hummulus lupulus* var. Brewer's Gold: Ultrastructural aspects of peltate trichomes. J Submicros Cytol Pathol 22: 241-248
Skocibusic M, Bezic N, Dunkic V (2006) Phytochemical composition and antimicrobial activities of the essential oils from *Satureja subspicata*. Food Chem 96: 20-28
Stephen O, Duke R, Paul N (1993) Development and fine structure of the glandular trichomes of *Artemisia annua* L. Intl J Plant Sci 154: 107-118
Turner GW, Gershezon J, Croteau RB (2000) Development of peltate glandular trichomes of peppermint. Plant Physiol 124: 665-679
Venkatachalam KV, Kjonaas R, Croteau R (1984) Development and essential oil content of secretory glands of sage (*Salvia officinalis*). Plant Physiol 76: 148-150
Wagner GJ (1990) Secreting glandular trichomes: More than just hairs. Plant Physiol 96: 675-679
Werker E (1993) Function of essential oil secreting glandular hairs in aromatic plants of Lamiaceae: A review. Flav Frag J 8: 249-255
Werker E, Putievsky E, Ravid U (1985) Essential oils and glandular hairs in different chemotypes of *Origanum vulgare* L. Ann Bot 55: 793-801
Werker E, Putievsky E, Ravid U, Dudai N, Katzir I (1993) Glandular hairs and essential oil in developing leaves of *Ocimum basilicum* L. Ann Bot 71: 43-50
Wise ML, Croteau R (1999) Monoterpene biosynthesis, *In* DE Cane, ed, Comparative Natural Products Chemistry, Vol 2. Elsevier Science, Oxford, pp 97-153