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## OSMOPHORES OF STANHOPEA (ORCHIDACEAE)<sup>1</sup>

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### ABSTRACT

Species of the Neotropical orchid genus *Stanhopea* produce a fragrance comprising terpenoids and aromatics which attracts euglossine bee pollinators. The secretory tissue, called an osmophore, is located in the adaxial region of a sac formed near the proximal portion of the floral lip. This region is easily recognized in *Stanhopea oculata* and *S. wardii* because it is papillate. The osmophore in these two species includes all the cells of the papillae and those directly below, that grade into fundamental tissue. Osmophore cells are more densely cytoplasmic than cells in the adjacent tissue. Numerous amyloplasts and mitochondria are seen in these cells from the earliest bud stages we examined through anthesis. Smooth and rough endoplasmic reticulum are abundant, but dictyosomes are uncommon. Mitochondria of osmophore cells appear to be distributed with no apparent pattern during bud stages, although they tend to be aligned near the plasmalemma at anthesis. Osmophore cells are highly vacuolate after anthesis.

*STANHOPEA* Frost ex Hooker is a Neotropical genus of Orchidaceae consisting of about 50 species. Members of *Stanhopea* are epiphytic herbs with ovoid or subcylindric, ribbed, unifoliate pseudobulbs (Fig. 1a). Leaves are plicate, elliptic to narrowly elliptic, conspicuously nerved, and distinctly petiolate. Inflorescences are sharply pendent from the base of the pseudobulb (Fig. 1–3) and bear 2–15 large, showy, short-lived, intensely aromatic flowers (Fig. 1, 4). The median sepal is coriaceous, erect, and free; lateral sepals are connate at their lower margins and adnate to the lip. Petals are narrower and less coriaceous than the sepals, and usually are reflexed strongly between them. The lip (Fig. 1b, 4, 5) is thick, fleshy, glossy, and usually divided into a basal, saccate hypochile, a mesochile bearing conspicuous horns, and a flattened, distal epichile. The fragrance emitting portion of the flower, or osmophore, of *S. oculata* and *S. wardii* consists of a papillate layer of tissue situated in the pouch of the hypochile (Fig. 1b, 5, 6). The column is elongate, usually broadly winged, and arched toward the lip. The anther is terminal with two pollinia.

*Stanhopea* is one of several Neotropical orchid genera pollinated by euglossine bees. There are published reports of the biology of these plants and their pollinating bees, of the chemistry of the compounds (mostly terpenoids and aromatics; Williams and Whitten, 1983) comprising the fragrances emitted by the flowers, and of the interactions between bee and flower. Dodson et al. (1969) and Dressler (1968, 1982) have studied the euglossine-bee-pollination syndrome in orchids. They reported that only male bees are associated with pollination, and that bees are attracted to the flowers by the strong fragrances they collect, presumably as a precursor for a sex pheromone they synthesize.

Osmophore structure has been studied in orchids with light microscopy by Vogel (1963), with scanning electron microscopy by Williams (1983), and with both scanning and transmission electron microscopy by Pridgeon and Stern (1983, 1985). The term osmophore (*osmo* from Gr. *osmo*, odor, and *pherein*, to bear) was first used by G. Arcangeli (1883) who applied it to the fragrant spadix of certain members of the Araceae (e.g., *Dracunculus vulgaris*, *Amorphophallus rivieri*). The external morphology of the osmophores in *Stanhopea* and other euglossine-bee-pollinated taxa varies from the numerous mounds of tissue in *Clowesia* and the trichomes of *Polycynis* to the papillae in some species of *Stanhopea* and smooth tissue of other species. Williams (1983) noted that in this group of orchids "the structure of the osmophore region is quite variable from species to species, and from genus to genus."

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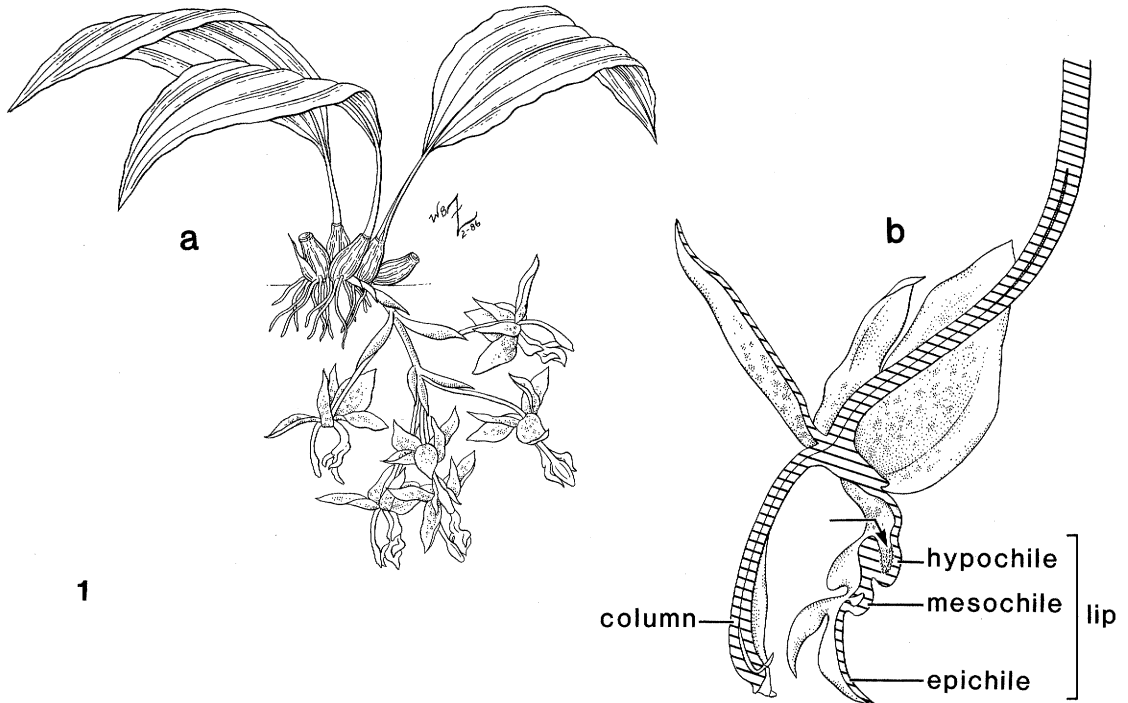


Fig. 1. a. Habit of a representative *Stanhopea* plant. b. Morphology of a typical *Stanhopea* flower (bisected) with the osmophore (arrow) located in the hypochile of the lip.

The objectives of this study are to describe the development of floral tissues comprising the osmophore in *Stanhopea* before, during, and following anthesis; to suggest sites of synthesis for fragrance compounds in the cells; to postulate the means by which these compounds pass from producing cells to the external tissue surface where they volatilize; and to provide a fundamental basis for future comparisons of fragrance production and osmophore structure among species of *Stanhopea* and other orchids.

**MATERIALS AND METHODS**—This study is based on *S. wardii* Lodd. ex Lindl., which ranges from Mexico through Central America to Venezuela, and *S. oculata* (Lodd.) Lindl., which occurs in Mexico and Central America (Dodson, 1963). These two species are morphologically similar and considered to be related (Dodson, 1963). No significant differences in osmophore structures were recognized, so the species will be treated as one complex in this paper. Several plants of each species, which were used for this study, are maintained in the greenhouses of the Department of Botany, University of Florida, and in the private collection of the first author.

Tissues for study were sampled at several developmental stages of the inflorescence. Buds

were appressed to the axis of the raceme, and little or no odor was evident during early stages of development (Fig. 2). Osmophore tissue was taken at this time from buds 3–4 cm long in *S. wardii* and 3.5–5 cm long in *S. oculata*. One or two days before the buds opened, the pedicels spread and moved the buds away from the axis of the inflorescence (Fig. 3). Osmophore tissue was collected at this stage from moderately fragrant buds, 4–5 cm long in *S. wardii* and slightly longer in *S. oculata*. Flowers remained open for only three days. Osmophore tissue from flowers of both plants was collected on the morning and at night during the first or second day. Tissue of postanthesis flowers (fourth day after opening) of *S. wardii* was also collected. The region sampled in all buds and flowers was the papillate layer in the hypochile region of the lip (Fig. 1b, 6). This region is the osmophore in *S. oculata* and *S. wardii*.

Material was fixed for two hr in 2% (w/v) formaldehyde (freshly made from paraformaldehyde), 2.5% (v/v) glutaraldehyde, and 2 mM  $\text{CaCl}_2$  in 0.1 M cacodylate buffer made to pH 7.2; buffer rinsed (0.1 M cacodylate, pH 7.2), postfixed for 45 min in 1% (w/v) osmium tetroxide in 0.1 M cacodylate buffer (pH 7.2), buffer rinsed (0.1 M cacodylate, pH 7.2), and dehydrated through an ethanol series (50, 70, 85, 95, 100%) followed by 100% acetone.

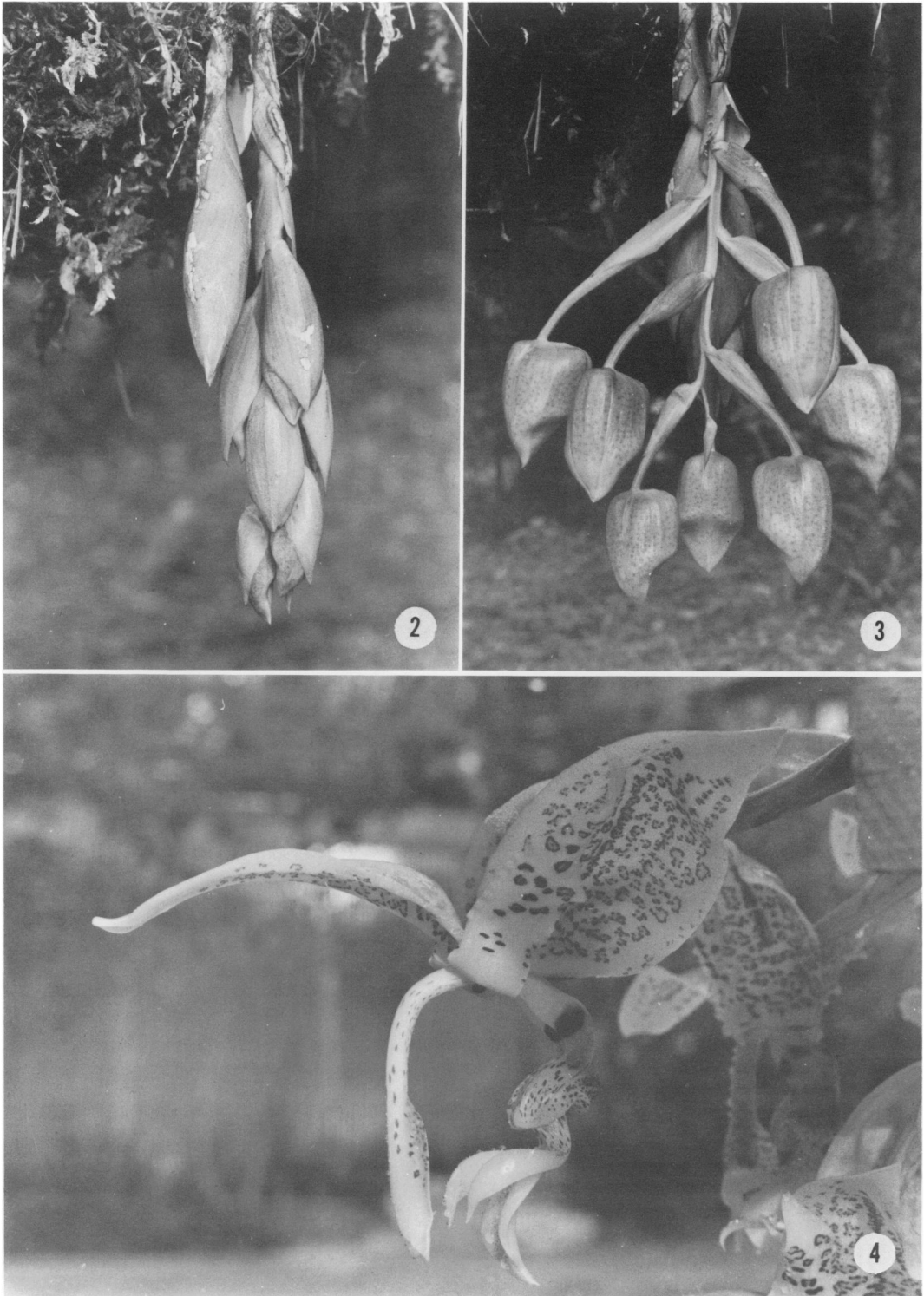


Fig. 2-4. Development of *Stanhopea* inflorescence. 2. Buds of *S. wardii*, pedicels appressed. 3. Buds of *S. wardii*, pedicels spread. 4. Flower of *S. oculata*.



Fig. 5, 6. The osmophore of *Stanhopea*. An asterisk at the tip of each hypocyle marks the equivalent point in both figures for orientation. 5. Flower of *S. wardii*. 6. Bisected hypocyle of *S. oculata* showing the papillate osmophore surface (arrows).

Materials for light microscopy and transmission electron microscopy (TEM) were embedded in epoxy resin ERL 4206 (Spurr, 1969). Material for light microscopy was thick-sectioned (ca. 1  $\mu\text{m}$ ) and stained with methylene blue-azure II-basic fuchsin (Humphrey and Pittman, 1974). Material for TEM was thin sectioned (ca. 90–100 nm), stained 30 min in 1% (w/v) aqueous uranyl acetate, and post-stained 15 min in Sato's lead citrate (Hayat, 1981). Dehydrated material for scanning electron microscopy was critical point dried and gold coated.

**RESULTS**—The bud stage in which the pedicels were appressed to the central axis and the bud stage in which the pedicels were spread represented two points in time several days apart that were distinguished easily, but there were no evident cellular changes between these two stages. There were, however, features to distinguish the bud stages from anthesis at both the light microscopic and ultrastructural levels, and these are described below.

*Light microscopy*—The surface of the osmophores is rugose and papillate. This aspect is compared in Fig. 7 and 8 (also cf. Fig. 6). Osmophores in both species of *Stanhopea* consist of several layers of cells characterized by moderate to dense cytoplasm, abundant amyloplasts, and lipid droplets. Cells farther from the center of glandular activity have increasingly larger vacuoles than those near to or part of the epidermis. These cells are presumably less active in the secretory process than either epidermal or immediately subtending cells (Fig. 8). All cells associated with the osmophore have a single nucleus containing a single nucleolus. The nuclei of osmophore cells appear larger than those of nearby cells (Fig. 8). Epidermal and subtending cells on the abaxial surface, which were not studied ultrastructurally, appear to be moderately secretory because they are cytologically similar to the most vacuolate cells of the adaxial, secretory tissue (Fig. 8). Both groups of cells have abundant amyloplasts and large vacuoles.

Starch grains are abundant in cells of the

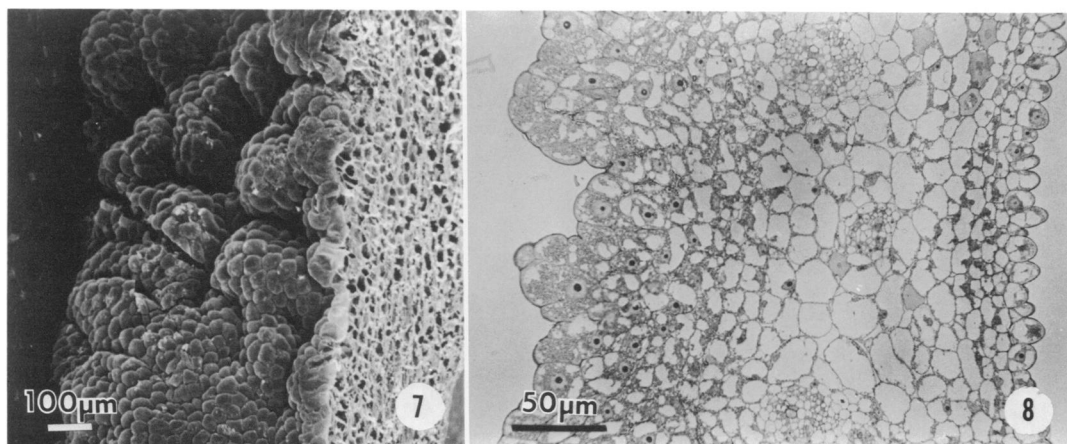


Fig. 7, 8. The osmophore of *Stanhopea*. 7. Scanning electron micrograph of the osmophore surface of *S. wardii* showing the varied morphology of papillae and rugae. 8. Section through the osmophore of *S. wardii*. The adaxial surface is to the left.

osmophore at all stages examined except post-anthesis (Fig. 9, 10). Osmophore cells are slightly to moderately vacuolate during bud stages and at anthesis (Fig. 9), but they are highly vacuolate after anthesis (Fig. 10). Lipid droplets are present in osmophore cells from bud stages through anthesis (Fig. 9).

*Scanning electron microscopy*—The osmophore area comprises numerous papillae of various sizes (Fig. 7). The cuticle covering the tips of most papillae is composed of convoluted ridges from the earliest stages examined, whereas the cuticle in “valleys” between the papillae is less ridged (Fig. 11). Occasionally a smooth-tipped papilla was observed (Fig. 12).

*Transmission electron microscopy*—For purposes of this paper, we refer to the epidermal wall exposed to the environment as “outer wall,” the wall of any cell (including the epidermis) separating two cells as “inner wall,” and, when used without modification, the term “wall” applies to both inner and outer walls.

Observations were limited to the epidermis and subtending three or four cell layers of the osmophore. The morphology and cytology of all cells within this region are essentially the same, except for the thicker, outer wall and cuticle of the epidermal cells. Mitochondria and amyloplasts with multiple starch grains are abundant in the osmophore cells at all stages of development (Fig. 13–15). Both smooth endoplasmic reticulum (SER) and rough endoplasmic reticulum (RER) occur in osmophore cells at all stages of development (Fig. 16, 17), but RER seems to be the common form in the

bud stages (Fig. 16). Dictyosomes, while not common at any time, were seen most often in the bud stages (Fig. 18).

Various places between the plasmalemma and the walls contain vesicles and amorphous material, but the phenomenon is more common in cells at anthesis than during bud stages (Fig. 17, 19, 20). Small globules or irregular areas of osmiophilic material, presumably lipid, are present in the cytoplasm of the bud stages and at anthesis (Fig. 14, 15, 17, 21), although the largest globules occur in cells at anthesis.

Mitochondria are distributed with no apparent pattern in osmophore cells during the bud stages (Fig. 13, 14). At anthesis, whether during the day or at night, the mitochondria cluster near one or more walls of various osmophore cells (Fig. 15). They may even align along portions of walls. However, there is apparently no correlation between the arrangement of mitochondria in one cell and those in an adjacent cell, nor does the polarity of the cell correlate with the alignment of mitochondria. Microbodies infrequently are intermingled with mitochondria (Fig. 21).

The amyloplasts contain starch grains, grana, and plastoglobuli at all stages of development (Fig. 22, 23). The plastoglobuli are frequently associated with the grana.

Processes of the outer epidermal cell wall extend into the cuticle at all stages of development (Fig. 24). Although extracuticular material is present at all stages, it is most common and forms a thicker layer at anthesis (Fig. 24).

We also examined the ultrastructure of osmophores from material fixed at night when fragrance was substantially weaker. No differ-

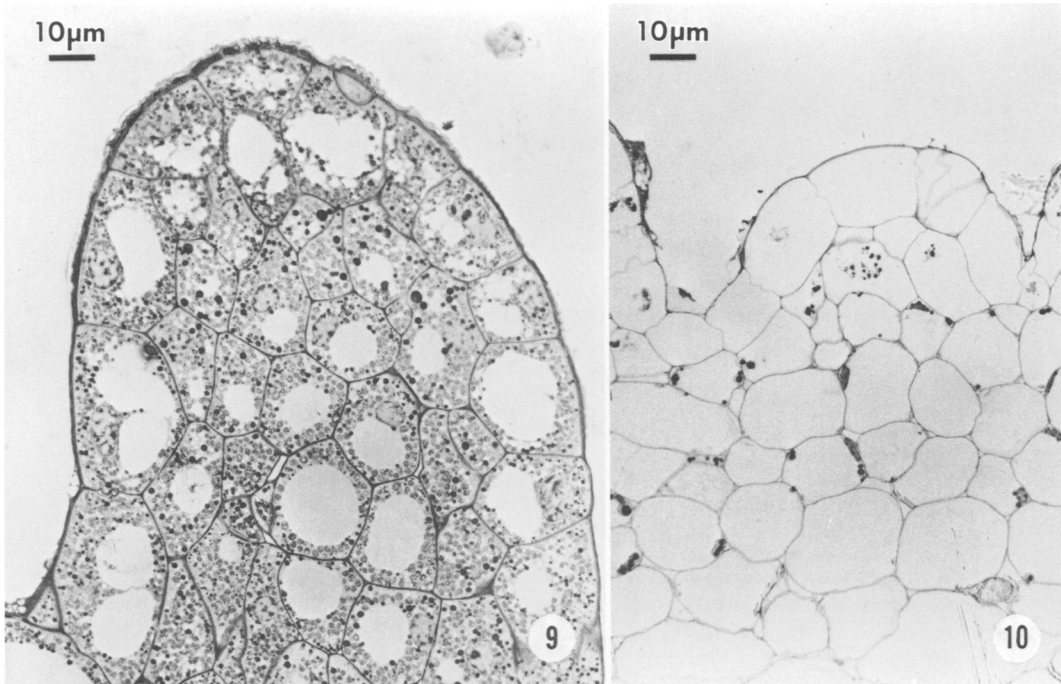


Fig. 9, 10. Osmophore tissue of *Stanhopea wardii*. 9. Tissue at anthesis. Each cell contains lipid droplets (black spheres) and numerous starch granules (gray spheres). 10. Vacuolate tissue at postanthesis.

ence in ultrastructure could be related to the obvious diminution of fragrance.

**DISCUSSION**—The starch grains present in osmophore tissue from bud stages through anthesis are an obvious source of carbon and energy for fragrance production, but their number does not appear to change between bud and anthesis even though fragrance is being synthesized actively. Fragrance glands of flower petals in general have been described as being

energy rich and provided with a constant supply of sugars from the phloem (Loomis and Croteau, 1973). Orchid flowers should represent no exception, and it might be this external carbon/energy supply from the phloem that is used for fragrance synthesis. Fragrance production is extremely low on the third (and final) day of anthesis (H. G. Hills, Florida State Museum, personal communication, based on chromatographic studies following the proce-

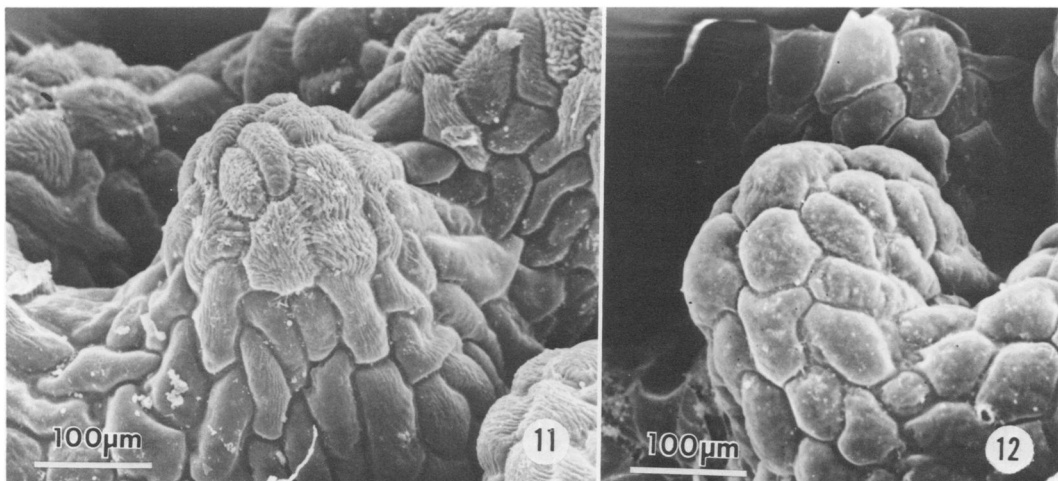


Fig. 11, 12. Scanning electron micrographs of osmophores of *S. wardii*. 11. Typical papilla. 12. An uncommon papilla tip with a smooth surface.



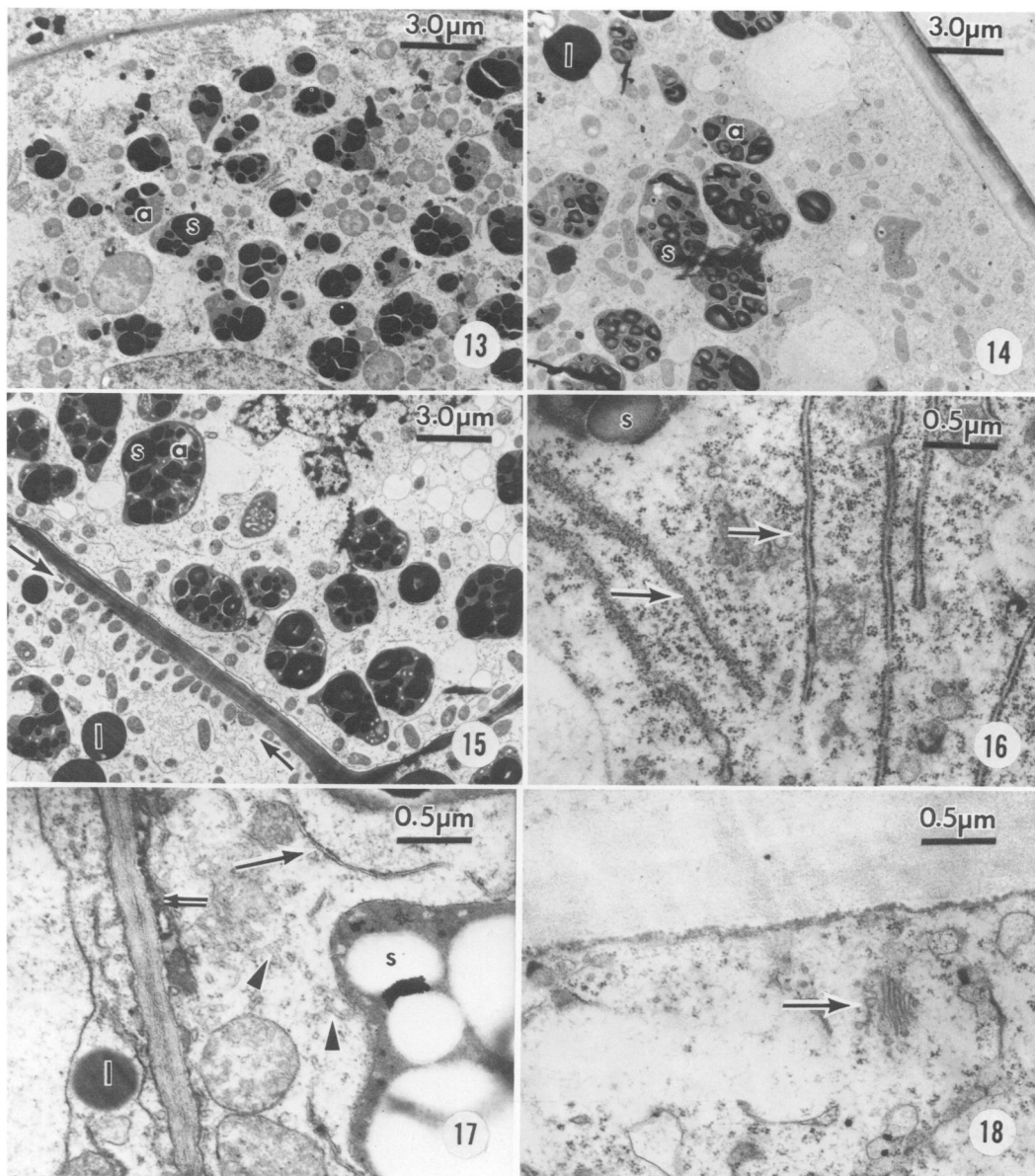


Fig. 13–18. Transmission electron micrographs of osmophore cells. a = amyloplast; l = lipid droplet; s = starch grain. 13. Bud of *S. wardii*, pedicels appressed. 14. Bud of *S. wardii*, pedicels spread. 15. *S. wardii* at anthesis. Mitochondria aligned along one wall (arrows). 16. Bud of *S. wardii*, pedicels appressed. Note rough endoplasmic reticulum (arrows). 17. *S. oculata* at anthesis. Smooth endoplasmic reticulum (arrowheads) and rough endoplasmic reticulum (arrow), amorphous material outside plasmalemma (double arrow). 18. Bud of *S. wardii*, pedicels appressed. Note dictyosome (arrow).

dures of Williams and Whitten, 1983). The starch is quickly mobilized on this day and is absent from osmophore cells on the fourth day when the flower has withered. This phenomenon whereby osmophore cells maintain a large energy/carbon supply in the form of starch during a period of presumably high metabolic activity (fragrance synthesis) and then mobilize this reserve just prior to senescence is curious and unexplained.

The accumulation of SER in osmophore cells at anthesis is typical for plant secretory cells (Schnepf, 1969). Smooth ER is associated with lipid secretion in general (Chrispeels, 1980) and with terpenoid biosynthesis in particular (Brooker and Russell, 1975). Dictyosomes, on the other hand, are not common in secretory cells of *Stanhopea*. Since they are generally associated with secretion of proteins, carbohydrates, and glycoproteins (Mollenhauer and

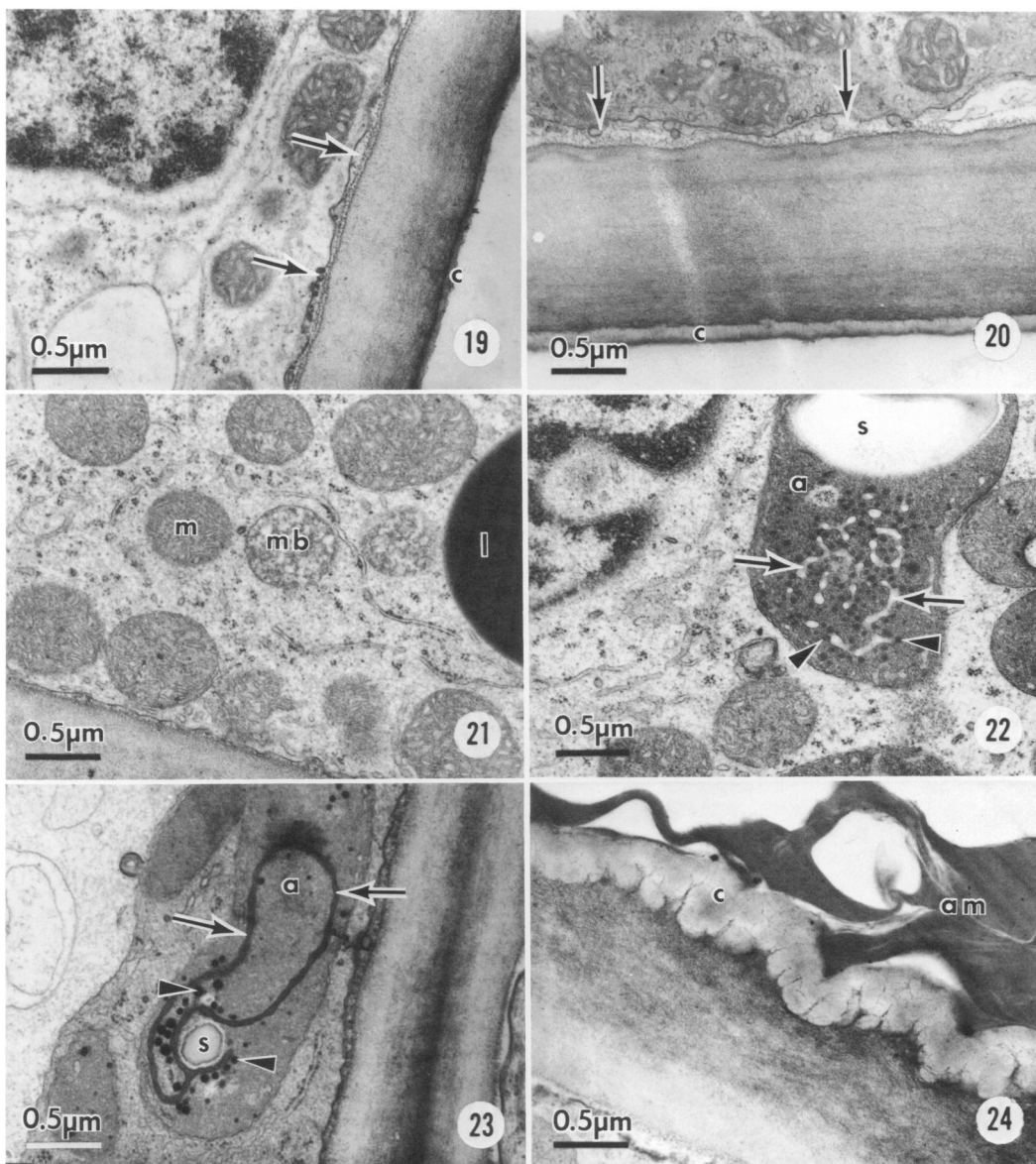


Fig. 19–24. Transmission electron micrographs of osmophore cells. a = amyloplast; am = amorphous material; c = cuticle; l = lipid droplet; m = mitochondrion; mb = microbody; s = starch grain. 19. Bud of *S. oculata*, pedicels spread. Amorphous material outside plasmalemma (arrows). 20. *S. wardii* at anthesis. Amorphous material and vesicles outside plasmalemma (arrows). 21. *S. wardii* at anthesis. Note lipid droplet and microbodies. 22. *S. wardii* at anthesis. Amyloplast with grana (arrows), associated plastoglobuli (arrowheads), and starch grain. 23. *S. oculata* at anthesis. Amyloplast with granum (arrows), associated plastoglobuli (arrowheads), and starch grain. 24. *S. oculata* at anthesis. Note amorphous material on surface of osmophore cell.

Morré, 1980), this is not a surprising observation.

The amorphous material between the plasmalemma and the cell wall of osmophore cells at all stages of development might be the fragrance mixture just before it enters the amyloplast. We did not see cytoplasmic vesicles fusing with the plasmalemma, so aromatic and terpenoid (lipid) fragrance molecules appar-

ently cross that membrane as individual molecules. We saw no evidence of aggregations of material moving through the apoplast as reported in *Restrepia* (Pridgeon and Stern, 1983), nor did we observe evidence of osmiophilic globules physically rupturing the cuticle of the osmophore cells as noted in *Restrepia*. An electron-dense material on the outer surface of the cuticle is present at anthesis, and may include

the fragrance compounds. Fragrance compounds are known to form an exudate on osmophore surfaces of some orchids (Vogel, 1963; Williams, Whitten, and Pedrosa, 1985).

Reasons for the redistribution of mitochondria from a random organization to positions aggregated along secretory cell walls at anthesis are unknown. Mitochondria might provide energy (adenosine triphosphate) for transport systems in the plasmalemma involved in fragrance secretion, and/or they might be involved in synthesis of the terpenoid component of the fragrance (Hemming, 1977).

The weak odor detected at night suggests the synthesis of fragrance is slowed or stopped then. The persistence of aroma, though, might be due to a gradual volatilization of fragrance thought to accumulate on or in the osmophore cells during the day. No ultrastructural changes associated with the diminution of fragrance production were observed.

Both species of *Stanhopea* produce a mixture of aromatics and terpenoids that include different ratios of different specific compounds (Williams and Whitten, 1983). The aromatic components are derived from the shikimic pathway that operates in microbodies and the cytosol (Rothe, 1974) and in plastids (Feierabend and Brasel, 1977). Plastids, in the form of amyloplasts, are common in osmophore cells, and microbodies are present in small numbers as well. Terpenoids are synthesized from mevalonic acid which is produced in plastids, mitochondria, and ER (Brooker and Russell, 1975). The large number of plastoglobuli in the amyloplasts of osmophore cells of *Stanhopea* suggests active secretion by those organelles. Goodwin (1977) and Grumbach and Forn (1980) indicated that regulation of the terpenoid pathways might be achieved by partitioning of the precursor pools into chloroplastidic and extraplastidic pools. Therefore, some organelles that produce terpenoids, e.g., amyloplasts, may not be solely involved in fragrance production. Cytochemical localization at the ultrastructural level of any enzymes specifically involved in terpenoid production would demonstrate which organelles are actually involved in terpenoid and, possibly, fragrance synthesis.

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