

## Secretory tissues of the flower of *Sanango racemosum* (Gesneriaceae). I. Light microscopy

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### SUMMARY

*Sanango racemosum* (Ruiz & Pav.) Barringer has a dry stigma without a free-flowing secretion fluid but with a hydrated proteinaceous pellicle. The stigmatic surface is covered with unicellular, bottle-shaped papillae. At maturity, a viscous emulsion is accumulated between the cuticle and the pecto-cellulosic wall of the papillae, causing it to become detached from the surface of the papilla cell walls. The style has a central solid core of transmitting tissue. The cells of the transmitting tissue are rich in starch and exhibit thick lateral walls rich in pectic substance. The nectary disk is a ring elongated into a cup, with five lobes at the top. One of the most conspicuous histological features of the disk is the abundance of starch in the secretory cells. The disk is supplied only by phloem; the stomata are found in the top of the lobes. A fluid substance is produced just before anthesis and secreted through the stomata with no visible decline in starch level. During anthesis and after fertilization, a rapid decline in starch is observed. The hypothesis that the disk has other functions besides that of a nectary is discussed.

*Key-words:* disk, nectary, osmophore, *Sanango*, stigma, transmitting tissue.

### INTRODUCTION

The monotypic genus *Sanango* G. S. Bunting and J. A. Duke is related to the Scrophulariales but what the most satisfactory family assignment of the genus should be remains to be seen. On the basis of the anatomical, morphological, embryological and chemical evidence, *S. racemosum* (Ruiz & Pav.) Barringer appears to be closely related to the Gesneriaceae but also shares important embryological features with the Scrophulariaceae (Dickison 1994; Jensen 1994; Maldonado *et al.* unpublished; Norman 1994; Wiehler 1994). Most families of the order Scrophulariales present an annular nectary-disk around the base of the ovary. Wiehler (1994) reported that the pronounced development of a nectary with the nectariferous tissue elongated into a cup as seen in the flowers of *Sanango* is not found in the Scrophulariaceae, nor in any other family of the Scrophulariales, except in the genus *Anetanthus* of the Gesneriaceae. In any case, at present, little is known about the secretory tissue of this annular nectary disk.

The morphological characteristics of the stigma have scarcely been accorded any significance in taxonomic and phylogenetic studies of the Scrophulariales. It seems that they could not be added to the list of criteria to be taken into account when classifying

the order Scrophulariales since wet and dry, papillate and non-papillate stigmas have been described in families of the order, including Scrophulariaceae and Gesneriaceae (Dumas 1975; Weber & Westphal 1978; Heslop-Harrison & Heslop-Harrison 1981).

This is the first histological study of cup-shaped disk and stigmatic-transmitting tissues of *S. racemosum*. A taxonomic comparison of these structures is impossible at present because of a lack of analogous studies of genera in families to which *Sanango* may be related. Much remains to be known about the ultrastructure of the tissue and composition of the secretion. We propose to take up these topics in further papers.

## MATERIAL AND METHODS

*Source of material.* *Sanango racemosum*: Ecuador, Napo: Jatun Sacha, 21 Sep 1990, Neill 9458 (DLF, MO, US).

*Specimen preparation.* Flowers fixed in FAA (formalin-acetic acid-ethyl alcohol) were embedded in paraffin and sectioned. Sections were cut at 8–10 µm and stained with iodine-potassium iodide and periodic acid-Schiff (PAS) (O'Brien & McCully 1981); fast green FCF (Fulcher *et al.* 1972); Sudan black (Bronner 1975); ruthenium red, Coomassie brilliant blue (Pearse 1985); acid fuchsin and toluidine blue O (Feder & O'Brien 1968).

## RESULTS

### *Secretory disk*

The disk is a ring elongated into a cup between stamen bases and ovary (Fig. 1a), and forming five lobes at the top. It consists of a specialized parenchymatous tissue covered by an epidermis without trichomes. The epidermis is composed of cells with dense cytoplasm and starch plastids. Starch was determined with iodine-potassium iodide and PAS. Stomata occur at the tops of the lobes and their guard cells also contain starch grains (Fig. 1c,d).

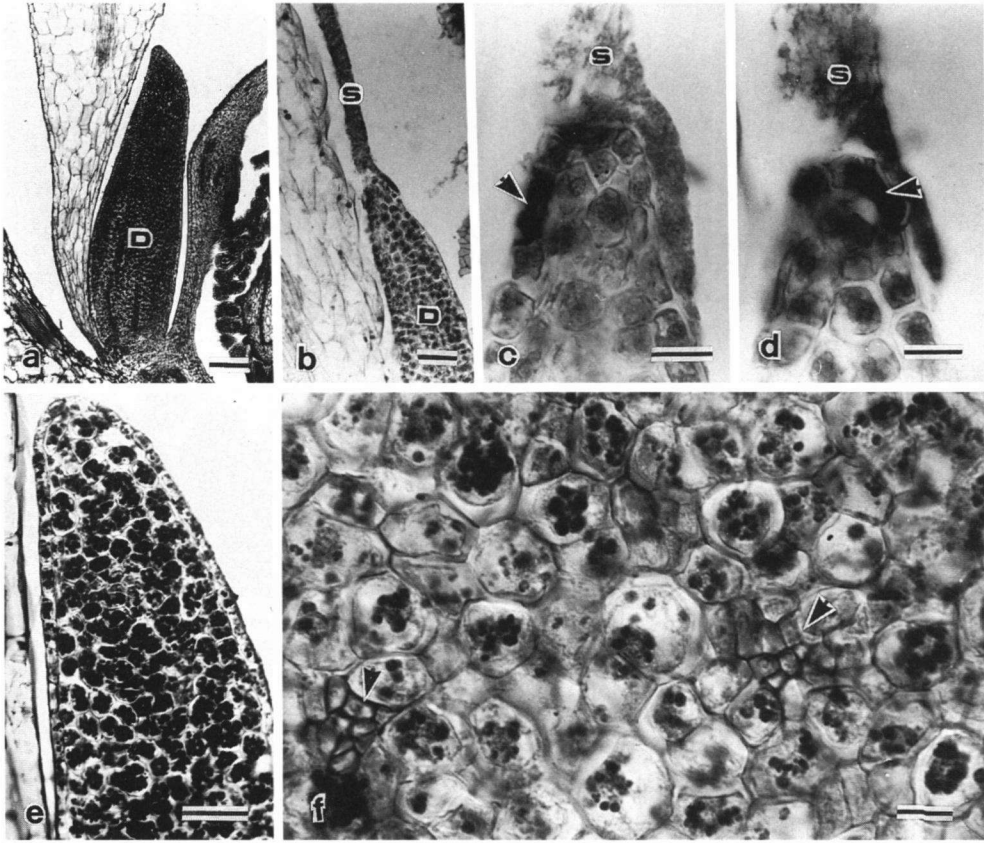
The secretory tissue is composed of small cells with thin walls, densely staining cytoplasm, small vacuoles and relatively large nuclei. Starch plastids are present in large amounts. Intercellular spaces are well defined in the parenchymatous tissue and a cuticular lining occurs along some intercellular spaces. Cell walls were stained with PAS, acid fuchsin and toluidine blue O and cutin was determined with Sudan black.

Synthesis of starch grains occurs during flower development and their number increases dramatically with the approach of anthesis (Fig. 1e). A fluid substance is produced just before anthesis (Fig. 1b) and is secreted through the stomata (Fig. 1c,d) with no visible decline in starch level. During anthesis and after fertilization, a rapid decline in starch is observed.

The disk is initially supplied by five traces composed entirely of phloem. The frequent branching of these results in the formation of a rich vascular system composed of a high number of bundles that extend acropetally into the disk. Phloem elements come into direct contact with the secretory cells (Fig. 1f).

### *Stigmatic and transmitting tissues*

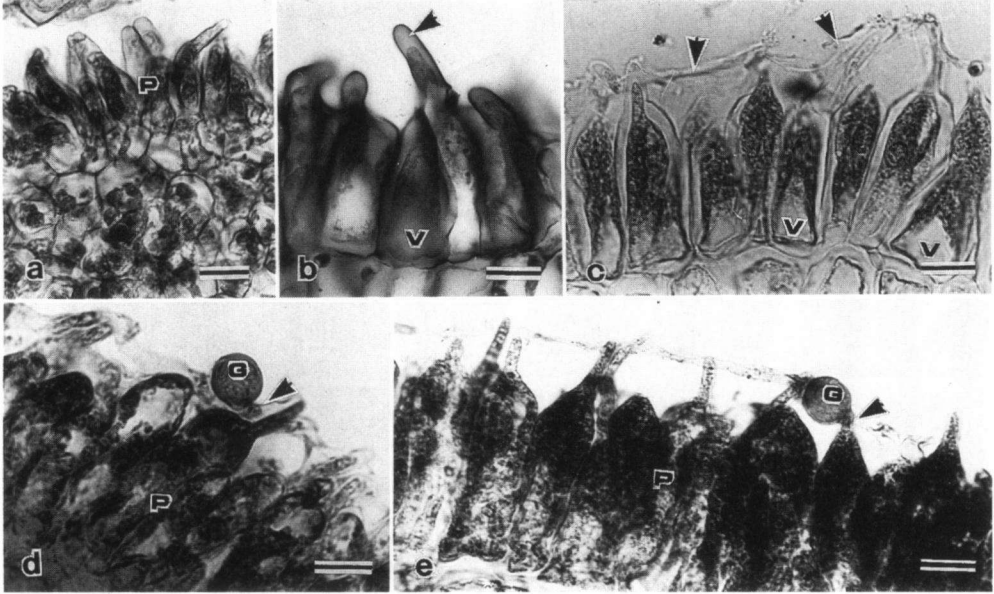
The stigma of *Sanango* is capitate with a bilobed head (Fig. 3a). The stigmatic surface is covered with unicellular bottle-shaped papillae, being the elongate tip of the receptive part of the papillae (Fig. 2b–e).



**Fig. 1.** (a) Secretory disk. Scale bar: 100  $\mu$ m (b) Disk secreting a fluid substance just before anthesis (stained with PAS reaction and fast green FCF). Scale bar: 100  $\mu$ m (c) and (d) Detail of the disk secreting the fluid substance through the stomata (arrows) (stained with PAS reaction and fast green FCF). Scale bar: 10  $\mu$ m. (e) Secretory tissue of the disk with abundant starch (positive iodine–potassium iodide and PAS reactions). Scale bar: 50  $\mu$ m. (f) Phloem bundles (arrows) in the secretory tissues of the disk. Scale bar: 10  $\mu$ m. D, disk; S, semi-liquid secretion.

Papillae are closely packed in young stigmas (Fig. 2a). During flower development, papillae separate and cuticle becomes demonstrable at the LM-level (Fig. 2b). A distinctive feature of these cells is the presence of numerous grains of starch. Such grains, single or compound, are iodine–potassium iodide and PAS positive. Cells of the subtending parenchyma also contain starch, but the grains are larger. In the papillate cells the cytoplasm stains densely with fast green and Coomassie blue. A relatively large vacuole is present at their base (Fig. 2b,c).

At maturity a very thin lipidic–proteinaceous pellicle, detected only after staining, overlies the cuticle. The protein was detected by staining with Coomassie blue and fast green FCF, and lipid by Sudan black. Later, a viscous emulsion accumulates between the cuticle and the pecto-cellulosic walls of the papillae, causing it to become detached from the surface of the cell walls (Fig. 2c). The cuticle is retained during pollination (Fig. 2d,e), suffering only partial disruption. No polysaccharides, lipids or proteins were present in the emulsion, as determined by a lack of staining with PAS, Sudan black and fast green FCF.



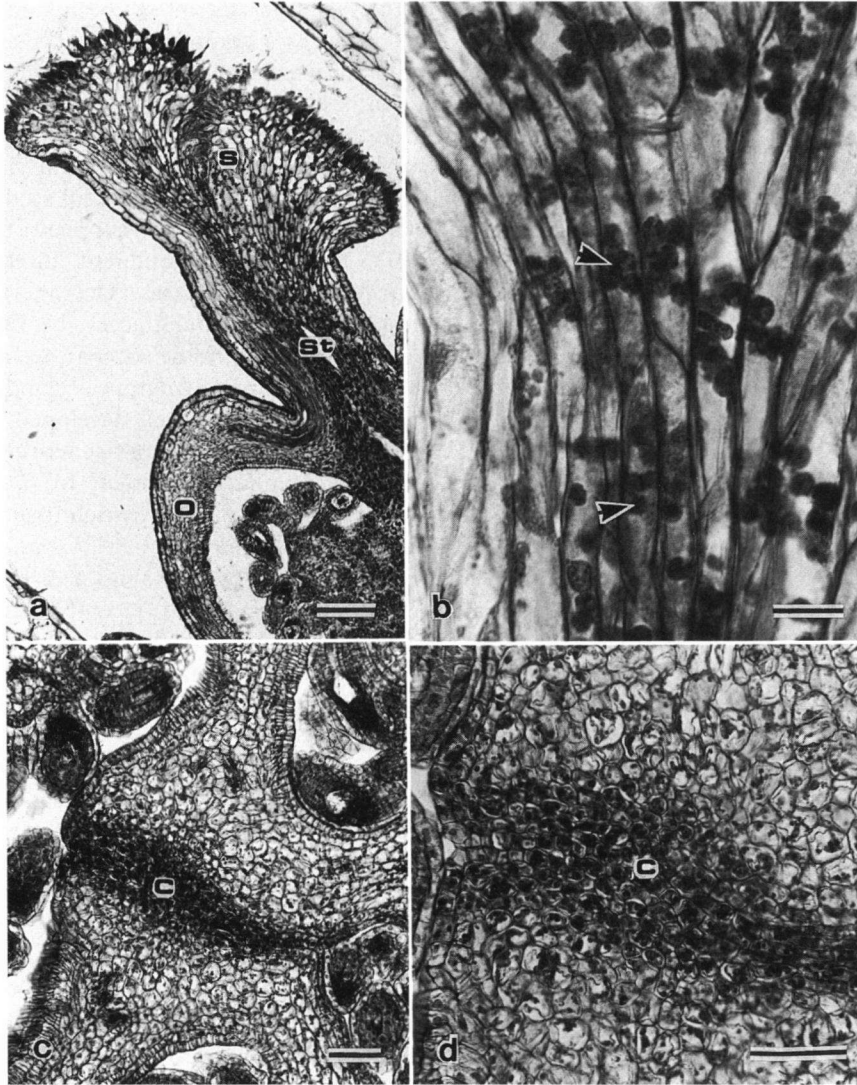
**Fig. 2.** (a) Young stigma with closely packed papillae. Scale bar: 10  $\mu$ m. (b) Separated papillae covered with a cuticle (arrow). Scale bar: 10  $\mu$ m. (c) Mature stigma with the detached cuticle (arrows). A very thin lipidic-proteinaceous pellicle overlies the cuticle (staining with Coomassie brilliant blue, fast green FCF and Sudan black). Large vacuoles are present in the papilla bases. Scale bar: 10  $\mu$ m. (d) and (e) Pollen grains adhered to the stigmatic surface producing tubes which penetrate the cuticle (arrows). Scale bar: 10  $\mu$ m. G, pollen grains; P, papillae; V, vacuoles.

The style has a central solid core of transmitting tissue. Cells of the transmitting tissue have some features in common with those of the stigma i.e. they have dense cytoplasm and are rich in starch (Fig. 3b). These cells exhibit thick lateral walls, rich in pectic substances, as determined with ruthenium red. The pollen tubes grow intercellularly through the outermost wall layer. The single strand of transmitting tissue is connected to the two placentae of the ovary constituting a *compitum* (Fig. 3c,d). (Latin: the open space where two or more ways meet, a crossing. According to Carr & Carr (1961), the eu-syncarpous gynoecium is characterized by the possession of a *compitum*, a connection between the carpels which allows the pollen tubes from grains germinating on any lobe of stigma to cross over to more than one loculus.)

Pollen grains adhere to the stigmatic surface. Those that germinate produce tubes which penetrate the cuticle (Fig. 2d,e) and grow towards the base of the papilla in the space occupied by the viscous emulsion. At the base of the papilla the tube continues to grow, moving intercellularly in the secretion produced by the subtending parenchyma and then in the transmitting tissue of the style. In the ovary, the pollen tubes go through the *compitum* then reach the secretory epidermis of the placenta. Pollen tubes are visible exiting the placenta, growing on the funiculus and penetrating the micropyle.

## DISCUSSION

According to Wiehler (1994) the cup-shaped disk of *Sanango* is a nectary. However, histological features, such as the abundance of starch in the secretory cells of the disk might indicate that the disk has other functions besides that of a nectary. Even though



**Fig. 3.** (a) Bilobed stigma, style and part of the ovary. Scale bar: 100  $\mu\text{m}$ . (b) Central, solid core of transmitting tissue in the style. Transmitting cells with starch grains (arrows) and thick lateral walls. Scale bar: 10  $\mu\text{m}$ . (c) and (d) The *compitum* connecting the two placentae (stained with PAS reaction and fast green FCF). Scale bar: 50  $\mu\text{m}$ . C, *compitum*; O, ovary; S, stigma; St, style.

in some plants, e.g. *Passiflora* (Dumas *et al.* 1981; Durkee 1983) and *Rosmarinus officinalis* (Zer & Fahn 1992), the amount of starch in the nectary of the flowers is very large and starts to decline as the nectary approaches the state of secretion, in almost all studies so far starch is present in a nectary only in small amounts that commonly disappear as secretion progresses (Durkee 1983). Conversely, cells rich in cytoplasm and filled with starch grains are associated with the osmophores which are restricted to certain areas of the flower organs, including nectaries (Fahn 1979; Roschina & Roschina 1993). Coincidentally, this statement is supported by an herbarium label (Dwyer 6228, from Tingo María, Peru) that notes that the flowers of *Sanango* are very fragrant

(Wiehler 1994). The chemical composition of the secretion has not yet been studied; if it were, it could perhaps reveal that the disk produces a combination of nectar and essential oils. The massive store of reserve material, which is utilized during emission, would provide the energy for the endothermic synthesis of the fragrance material as well as of building material. It should be emphasized that there are few direct experimental data confirming the existence of one or the other role of the disk in Scrophulariales.

Few descriptions of the nectar vascular tissue are available. In almost all studies so far, the vascular bundles consist of both phloem and xylem. Nectaries supplied entirely by phloem have been reported by Fahn (1979) for flowers of different families of Dicotyledons: Aceraceae, Crassulaceae, Cruciferae, Fumariaceae, Oenotheraceae, Polemoniaceae, Rhamnaceae, Rutaceae, Salicaceae and Scrophulariaceae, by Daphni *et al.* (1988) for flowers of the Labiatae, and by Figier (1971) in the stipular nectary of *Vicia faba*. According to Vogel (1990) the most compact fragrance glands are characterized by an intensive, chiefly phloemic venation and a well developed intercellular system with abundant stomata. The intense respiration during the active phase is generally correlated with the development of a ventilation system. In *Sanango racemosum*, the disk is supplied by phloem in direct contact with starch-rich tissue with many intercellular spaces; stomata are found at the top of the disk.

The style of *Sanango* accords to the DPU type in the Heslop-Harrison & Heslop-Harrison classification (1981) because: (a) stigma surface is dry; (b) receptive cells are concentrated in a head; (c) stigma surface is papillate; and (d) papillae are unicellular. According to Heslop-Harrison & Shivanna (1977), dry stigmas with papillate surfaces and unicellular papillae occur in genera belonging to Gesneriaceae (*Aeschynanthus*, *Columnea*, *Fieldia* and *Saintpaulia*) and Scrophulariaceae (*Anthirrinum*, *Chelone*, *Digitalis*, *Gratiola*, *Hebe*, *Lathraea*, *Linaria*, *Pentstemon*, *Scrophularia*, *Torenia*, *Verbascum* and *Veronica*) and multicellular papillae are present in *Alonsoa* (Scrophulariaceae). Nevertheless, it is impossible to assess the taxonomic significance of these characteristics because dry stigmas with non-papillate surfaces have been found in the genus *Veronicastrum* of Scrophulariaceae and wet stigmas with low to medium papillae have been reported in genera of those same families, i.e. *Sinningia* and *Streptocarpus* of Gesneriaceae and *Calceolaria* and *Nemesia* of Scrophulariaceae. The basic division into wet and dry types presents difficulties in some species where the stigma bears only a small amount of secretion (Heslop-Harrison 1981).

The morphology of the papillae in *Sanango* is arguably an odd phenomenon in the order Scrophulariales. Notwithstanding the taxonomic remoteness between *Sanango* and Iridaceae, the structure of the papillae with a wide base and a long, narrow receptive point are quite similar to those observed in *Crocus* by Heslop-Harrison (1977).

In *S. racemosum* the stigmatic cells secrete a viscous material beneath the cuticle, causing it to become detached from the outer surface of the stigmatic cell walls. This behaviour was observed in *Brassica oleraceae*, *Arabidopsis thaliana* and *Papaver rhoeas* by Elleman *et al.* (1992) and interpreted as one of the early stigmatic responses in species with dry stigmas to pollination. In *Sanango*, as in *Brassica* and *Papaver*, the pollen tubes then penetrate the cuticle and grow towards the base of the papilla in the space thus generated.

A pellicle overlies the cuticle of the stigma papillae of *S. racemosum*. The pellicle was first found in *Silene vulgaris*, *Brassica oleraceae* and *Raphanus sativus* and its presence confirmed in some 80 other angiosperm families, indicating that it may well be universal in the dry stigma group (Heslop-Harrison 1977; Heslop-Harrison & Heslop-Harrison

1980; Mattsson *et al.* 1974). Beyond its function in the capture and hydration of pollen, there is the possibility that the pellicle might be concerned in pollen–stigma interaction. The layer seems to be the site of the recognition reaction during pollination (Heslop-Harrison 1977).

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## REFERENCES

- Bronner, R. (1975): Simultaneous demonstration of lipids and starch in plant tissues. *Stain Technol.* **50**: 1–4.
- Carr, S.G. & Carr, D.J. (1961): The functional significance of syncarpy. *Phytomorphology* **11**: 249–256.
- Dafni, H., Lensky, J. & Fahn, A. (1988): Flower and nectar characteristics of nine species of Labiatae and their influence on honey bee visits. *J. Apicultural Res.* **27**: 103–114.
- Dickison, W.C. (1994): A re-examination of *Sanango racemosum*. 2. Vegetative and floral anatomy. *Taxon* **43**: 601–618.
- Dumas, C. (1975): *Le stigmaté et la sécrétion stigmatique. Étude histophysique et biochimique de l'activité glandulaire lipophile*. Thesis Dr d'état es sciences naturelles. Université Glaude Bernard, Lyon.
- Dumas, C., Gaal, D.J. & Reisner, W.H. (1981): The floral and extrafloral nectaries of *Passiflora*. 1. The floral nectary. *Am. J. Bot.* **68**: 453–462.
- Durkee, L.P. (1983): The ultrastructure of floral and extrafloral nectaries. In: Bentley, B. & Elias, T. (eds): *The Biology of Nectaries*. Columbia University Press, New York.
- Elleman, C.J., Francklin-Tong, V. & Dickinson, H.G. (1992): Pollination in species with dry stigmas: the nature of the early stigmatic response and the pathway taken by pollen tubes. *New Phytol.* **121**: 413–424.
- Fahn, A. (1979): *Secretory Tissues in Plants*. Academic Press, London.
- Feder, N. & O'Brien, T.P. (1968): Plant microtechnique: some principles and new methods. *Am. J. Bot.* **55**: 123–142.
- Figier, J. (1971): Etude infrastructurale de la stipule de *Vicia faba* L. au niveau du nectaire. *Planta* **108**: 215–226.
- Fulcher, R.G., O'Brien, T.P. & Simmonds, D.H. (1972): Localization of arginine-rich proteins in mature seeds of some members of Graminaea. *Aust. J. Biol. Sci.* **25**: 487–497.
- Heslop-Harrison, J. & Heslop-Harrison, Y. (1980): The pollen stigma interaction in the grasses. 1. Fine structure and cytochemistry of the stigmas of *Hordeum* and *Secale*. *Acta Bot. Neerl.* **29**: 261–276.
- Heslop-Harrison, J. & Heslop-Harrison, Y. (1981): The specialized cuticles of the receptive surfaces of angiosperm stigmas. In: Cutler, D.F., Alvin, K.L. & Price, C.E. (eds): *The Plant Cuticle*. Academic Press, London.
- Heslop-Harrison, Y. (1977): The pollen–stigma interaction: pollen tube penetration in *Crocus*. *Ann. Bot.* **41**: 913–922.
- Heslop-Harrison, Y. (1981): Stigma characteristics and angiosperm taxonomy. *Nordic J. Bot.* **1**: 401–420.
- Heslop-Harrison, Y. & Shivanna, K.R. (1977): The receptive surface of the angiosperm stigma. *Ann. Bot.* **41**: 1233–1258.
- Jensen, S.R. (1994): A re-examination of *Sanango racemosum*. 3. Chemotaxonomy. *Taxon* **43**: 619–623.
- Mattson, O., Knox, R.B., Heslop-Harrison, J. & Heslop-Harrison, Y. (1974): Protein pellicle of stigmatic papillae as a probable recognition site in incompatibility reactions. *Nature* **247**: 298–300.
- Norman, E.M. (1994): A re-examination of *Sanango racemosum*. 2. Vegetative and floral anatomy. *Taxon* **43**: 591–600.
- O'Brien, T. P. & McCully, M.E. (1981): *The Study of Plant Structure. Principle and selected methods*. Thermacarphy Pty. Ltd, Melbourne.
- Pearse, A.G.E. (1985): *Histochemistry, Theoretical and applied*, 4th edn. Churchill Livingstone, New York.
- Roschina, V.V. & Roschina, V.D. (1993): *The Excretory Function of Higher Plants*. Springer Verlag, Berlin.
- Vogel, S. (1990): *The Role of Scent Glands in Pollination*. Smithsonian Institution Libraries and The National Sciences Foundation, Washington.
- Weber, H.C. & Westphal, B. (1978): Spezielle Formen von Epidermiszellen an dem Alpenrachen *Tozzia alpine* L., Scrophulariaceae. Raster-Elektronmikroskopie (Zeiss) Anwendungsblatt Elo 4.

- Wiehler, H. (1994): A re-examination of *Sanango racemosum*. 4. Its new systematic position in Gesneriaceae. *Taxon* **43**: 625–632.
- Zer, H. & Fahn, A. (1992): Floral nectaries of *Rosmarinus officinalis* L. Structure, ultrastructure and nectar secretion. *Ann. Bot.* **70**: 391–397.