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Nectary structure in *Symphyglossum sanguineum* (Rchb.f.) Schltr. (Orchidaceae)

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S u m m a r y

Ornithophily occurs in a great number of orchid species but despite this, researchers have largely neglected to investigate their nectaries. The aim of this study is to describe the nectary structure of *Symphyglossum sanguineum,* a species presumed to be pollinated by hummingbirds. The nectary is located at the free margins of auricles, which form a channel for the passage of nectar. The nectary, which consists of a single-layered epidermis and 2-3 layers of subepidermal cells, is supplied by collateral, vascular bundles. The nectary cells of *S. sanguineum*, like those of other ornithophilous orchids, have thick cellulose cell walls. A remarkable feature of these nectary cells is the dissolution of the middle lamella and the subsequent separation of epidermal cells. It is possible that this latter process facilitates the flow of the nectar to the nectary surface. The cuticle covering the nectary epidermis has micro-channels, but unlike the other species of ornithophilous orchids studied to date, it neither becomes disrupted nor detached from the epidermal cells. Abundant mitochondria, lipid droplets and smooth endoplasmic reticulum (SER) with an osmiophilic material are present in the cytoplasm of nectary cells. Some plastids with few lamellae contain numerous vesicles and osmiophillic globules whereas others accumulate starch. SER lamellae are often closely associated with plastids and the contents of the former organelles closely resemble osmiophillic globules. Secretory vesicles are common, especially near the outer, tangential wall indicating that granulocrine secretion possibly occurs in *S. sanguineum*.

Key words: nectary, ornithophily, pollination, *Symphyglossum sanguineum*, Orchidaceae

INTRODUCTION

The floral diversity of orchids reflects both the pollination strategies employed and the type of reward offered to pollinators. Even so, a substantial number of orchids offer no reward whatsoever and attract pollinators by deception (Neiland and Wilcock, 1998; 2000), Conversely, those species that offer rewards may produce pseudopollen (van der Pjil and Dodson, 1969; Davies et al., 2002; 2003; Davies and Turner, 2004 a, b, c), wax or a viscid, resinous material secreted by the labellum (van der Pjil and Dodson, 1969; Davies et al., 2003), fragrant substances (Flach et al., 2004; Singer and Koehler, 2004), oils (Steiner, 1998; Reis et al., 2000) or floral nectar. Nectar is the most common food reward in orchids and represents the most effective way of ensuring successful pollination even when there is a paucity of potential pollinators (Neiland and Wilcock, 1995; 1998).

Morphologically the nectaries of orchids are very diverse. Frequently, the nectary is in the form of the spur that arises from one of the perianth segments as in *Calanthe*, *Comparettia*, *Disperis*, *Satyrium* and *Tipularia* (Dressler, 1990) or as an outgrowth from the proximal part of the labellum as in *Platanthera* (Stpiczyńska, 1997) or *Gymnadenia* (Stpiczyńska and Matusiewicz, 2001). In Spirantheae, the nectary is formed from the lateral sepals, the labellum and the column-foot (Singer and Sazima, 1999). Likewise, in *Hexisea imbricata* and *Systeloglossum,* the nectary is represented by a saccate spur formed by the fusion of the column and proximal part of the labellum (Dressler, 1990; Stpiczyńska et al., 2005). Many other orchids have nectaries located upon their labella (eg. *Bulbophyllum ipanemense*, *B. involutum*, *B. weddellii* (Teixeira et al., 2004), *Epipactis atropurpurea* (Pais, 1987) and *Maxillaria parviflora* (Singer and Koehler, 2004) whereas in *Maxillaria anceps*, nectar is secreted by the labellar callus (Davies et al., 2005). In the presumed ornithophilous orchid of *Maxillaria coccinea,* the nectary occurs as an outgrowth upon the ventral surface of the column (Stpiczyńska et al., 2004). Likewise, the nectary structure and the manner in which nectar is secreted can also be very diverse (Pais and Figueiredo, 1994; Stpiczyńska, 1997; Stpiczyńska and Matusiewicz, 2001; Stpiczyńska et al., 2004; 2005).

The aim of the present paper is to describe the nectary structure of the presumed ornithophilous orchid (van der Pijl and Dodson, 1969) *Symphyglossum sanguineum* and comparison with that of *H. imbricata* and *M. coccinea* would perhaps help us better understand the structural features that characterize the nectary of ornithophilous orchids.

MATERIALS AND METHODS

The position of the nectary in intact, fresh flowers of *Symphyglossum sanguineum* (Rchb.f.) Schltr. was determined using an Olympus SZX12 stereomicroscope. Hand-cut sections through the nectary were tested for starch and

lipids using IKI and a saturated alcoholic solution of Sudan III, respectively. Pieces of nectary tissue were fixed in 2.5% glutaraldehyde / 5% sucrose in phosphate buffer (pH 6.8; 0.075M) for 4h at 20° C, washed in phosphate buffer and post-fixed in 1% osmium tetroxide at 0° C for 2h. The fixed material was then dehydrated using a graded ethanol series, infiltrated and embedded in Spurr resin. For general histology, semi-thin sections (about 1 µm thick) were stained using Azure B or 1% (w/v) toluidine blue in 1% (w/v) aqueous sodium tetraborate solution. Micrometry and photomicrography of the nectaries were accomplished using a Nikon Eclipse 600 microscope with Screen Measurement version 4.21 software. TEM sections were cut at about 60 nm using a glass knife and a Reichert Om U-3 ultramicrotome. Sections were stained with uranyl acetate and lead citrate and examined using a TESLA BS-340 transmission electron microscope at an accelerating voltage of 60kV.

RESULTS

The racemose inflorescence of *Symphyglossum sanguineum* bears several pink-violet, scentless flowers. These show diurnal anthesis, are only weakly zygomorphic, produce copious nectar, seemingly lack nectar guides and have reflexed labella. The ovate, acute labellum is clawed and at its proximity bears two vertical auricles. Both labellum and auricles are adnate to the column (Fig. 1) and the opening between the two auricles is small and located close to the reproductive organs. The nectaries are located upon each of the auricles and nectar flows along the channel formed by the two auricles onto the surface of the lip (Fig. 2).

The nectary consists of a single-layered epidermis with few stomata and 2-3 layers of subepidermal cells (Figs 3, 4). The secretory cells are small (18.21 24.34 µm; mean = 21.71 µm) and have large nuclei and dense, granular cytoplasm (Fig. 5). Their small vacuoles, as seen using TEM, are electron- translucent or may contain raphides (Fig. 6). The nectary is supplied with large vascular bundles comprising both xylem and phloem elements (Fig. 4).

The tangential, cellulose walls of the secretory cells are thick (3.77 - 6.43 µm; mean = 5.18 µm) and numerous pits with associated plasmodesmata connnect the protoplasts of contiguous secretory cells (Fig. 7). Often, dissolution of the middle lamellae of radial walls results in the separation of the epidermal, secretory cells (Figs 8, 9, 10) and once separated, these cells may be displaced to varying degrees, but their outer walls always remain intact, at least as thin layer. The use of the metachromatic stain toluidine blue did not indicate the presence of lignin nor suberin within the walls of secretory cells. However, the outer, tangential walls have a thick cuticle with numerous micro-channels (Fig. 11). This cuticular layer remains intact, lacking surface pores and cracks, even at points where radial, epidermal cell walls have become separated (Fig. 10).

The cytoplasm of the nectary cells contains abundant mitochondria, dictyosomes and smooth endoplasmic reticulum (SER). The SER profiles are irregular in shape

Fig. 1. Nectary of *Symphyglossum sanguineum* upon the labellar auricles (arrow). Scale bar = 2 mm.

Fig. 2. Adaxial surface of the labellum showing the narrow gap between the auricles and nectar (arrow) upon the labellar surface. Scale bar = 3 mm.

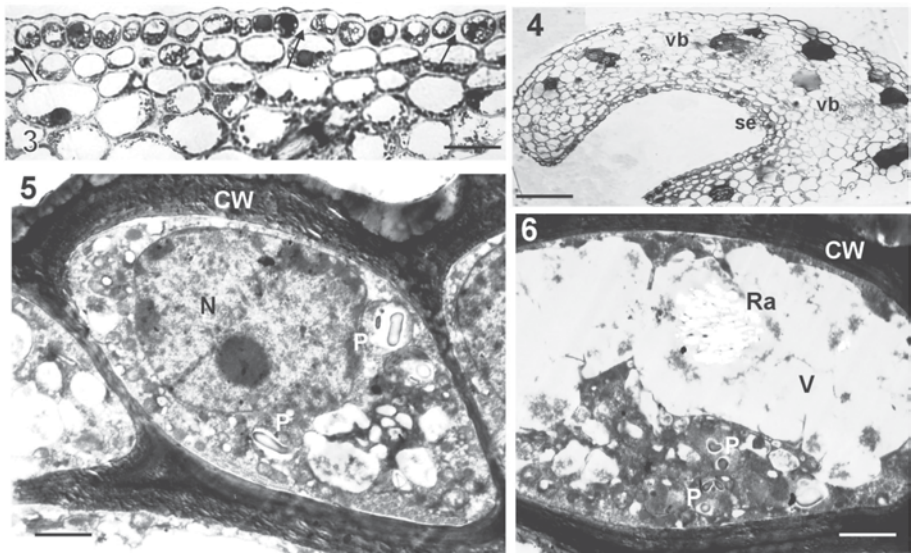
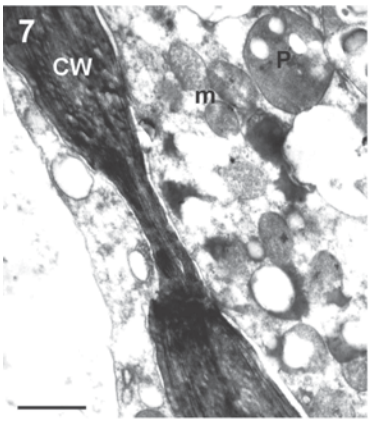


Fig. 3. Transverse section of nectary showing separation and displacement of some epidermal cells (arrows). Scale bar = 50 µm.

Fig. 4. Transverse section of nectary showing vascular bundles. Scale bar = 0.15 mm.

Fig. 5. Epidermal cell of nectary with nucleus, dense cytoplasm, plastids containing starch grains and thick, outer, tangential cell wall. Scale bar = 5 µm.

Fig. 6. Raphides within the vacuole of secretory epidermal cell. Scale bar = 5 µm.



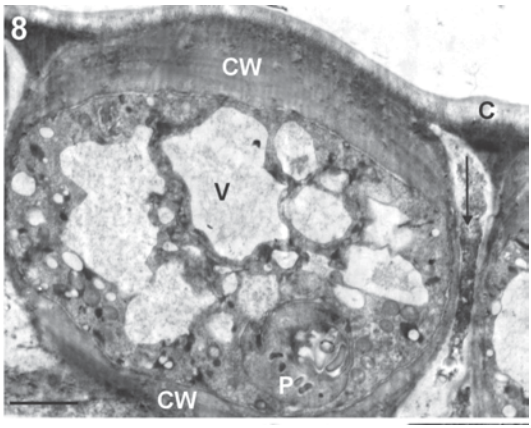
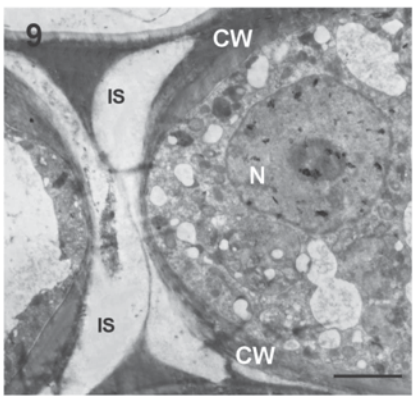
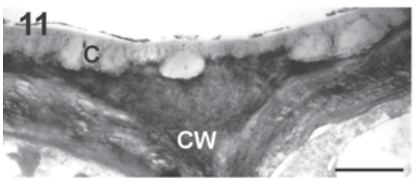


Fig. 7. Cell wall pit with plasmodesmata connecting contiguous nectary cells. Scale bar = 1.5 µm.

Fig. 8. Dissolution of the middle lamella between secretory epidermal cells (arrow). Scale bar = 5 µm.





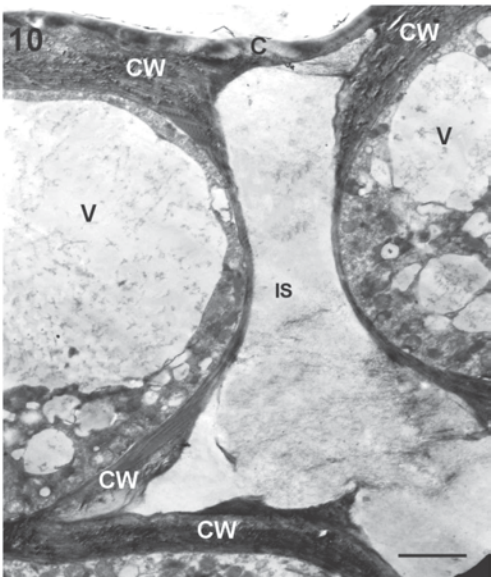


Fig. 9. Separation of secretory epidermal cells. Scale bar = 7.5 µm.

Fig. 10. Large, intercellular space between secretory cells. The outer, tangential cell wall and cuticle remain intact. Scale bar = 5 µm.

Fig. 11. Cuticle covering outer, tangential cell wall with micro channels. Scale bar = 3.5 µm.

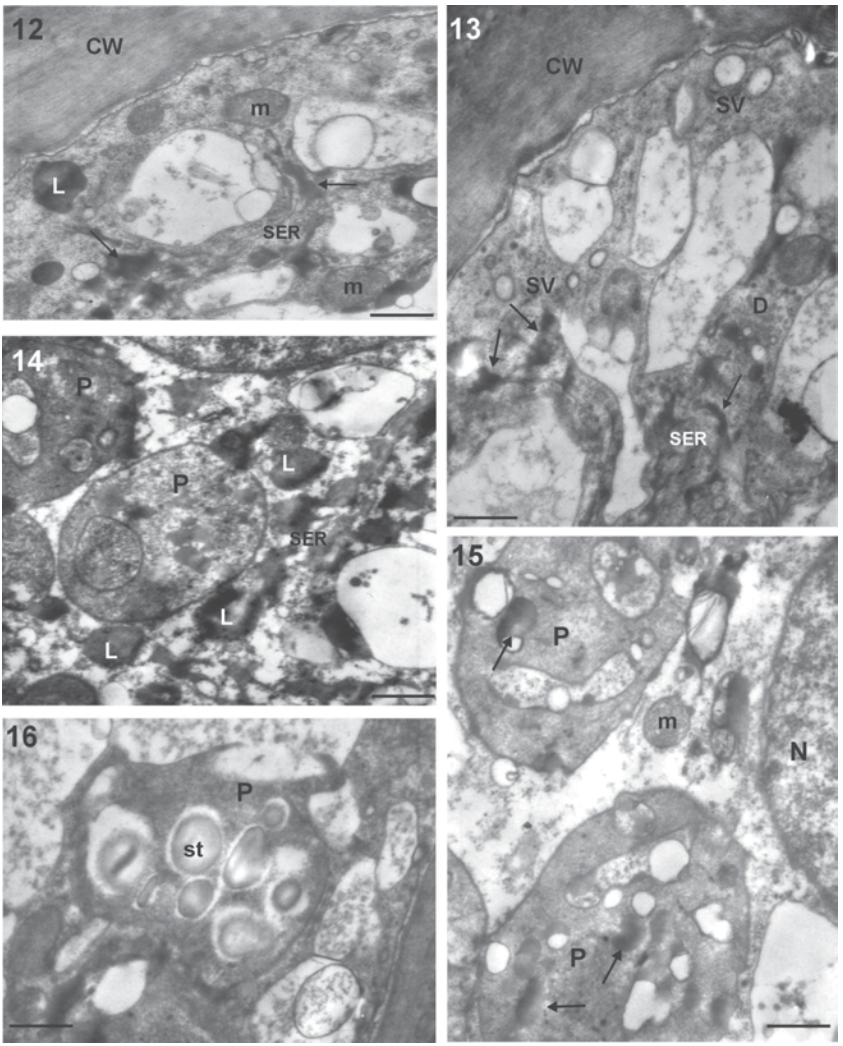


Fig. 12. Cytoplasm of secretory cell with irregular SER profiles that contain osmiophilic material (arrows) and lipid droplets. Scale bar = 2 µm.

Fig. 13. Secretory vesicles in close proximity to the cell wall. SER profiles with osmiophilic material indicated by arrows. Scale bar = 1.5 µm.

Fig.14. Cytoplasm of secretory cell with lipid droplets and SER profiles in close proximity to the plastids. Scale bar = 2 µm.

Fig. 15. Plastid with vesicles and osmiophilic globules similar to that found in the SER (arrows). Scale bar = 1.5 µm.

Fig.16. Amyloplast with starch grains. Scale bar = 1.5 µm.

Abbreviations: Ne nectary; Vb vascular bundle, Cw cell wall, C cuticle, N nucleus, SER smoth endoplasmic reticulum, M mitochondrion, P plastid, st starch, L lipid droplet, V vacuole, sv secretory vesicle, Ra raphides, IS intercellular space, se secretory epidermis

and contain osmiophilic material (Figs 12, 13). The cytoplasm also contains plastids, numerous lipid droplets and secretory vesicles that are common and often aggregate in large numbers adjacent to the cell wall (Fig. 13). SER membranes often become associated with plastids whose osmiophilic globules closely resemble the contents of the SER (Figs 14, 15). Some plastids contain abundant vesicles and osmiophilic globules whereas others enclose starch grains but few lamellae (Fig. 16).

DISCUSSION

The flowers of *Symphyglossum sanguineum* share a number of features with other presumed ornithophilous orchids such as *Maxillaria coccinea* (Stpiczyńska et al., 2004) and *Hexisea imbricata* (Stpiczyńska et al., 2005). They all show diurnal anthesis, are weakly zygomorphic, lack nectar guides and fragrance, produce copious nectar, have reflexed labella and a small opening at the level of the reproductive organs. Although the locations of the nectaries of the three former orchid species are very different, nectar would, nonetheless, be accessible to visiting hummingbirds.

In *S. sanguineum*, as in *M. coccinea* and *H. imbricata*, the nectary consists of a single-layered epidermis and small, subepidermal cells. In each case, the secretory cells have thick cell walls and this feature may prevent the nectary from becoming damaged by the beak of a visiting bird. The cell walls, which consist of cellulose, together with the cuticle, appear to be permeable to secreted nectar. A remarkable feature of the nectary cells of *S. sanguineum* is the dissolution of the middle lamella and the subsequent separation and displacement of epidermal cells. It is possible that these processes facilitate the flow of nectar onto the surface of the nectary. Such features were not observed in other ornithophilous species. Despite the scant occurrence of stomata upon the nectary of *S. sanguineum*, it is unlikely, that these are involved in nectar secretion, similarly as in *Maxillaria coccinea* (Stpiczyńska et al., 2004) and *H. imbricata* (Stpiczyńska et al., 2005). The epidermal cuticle of *S. sanguineum* has a similar structure to that of *H. imbricata* and *M. coccinea* but, unlike these two species, the cuticle of *S. sanguineum* does not become detached from the epidermis as nectar accumulates between it and the outer, tangential wall.

Transport of nectar in *S. sanguineum* is possible by means of plasmodesmata, which traverse the thick, cellulose, cell walls. Cell walls and intercellular spaces probably also play a part as the nectar passes along the apoplast. This, according to Vassilyev (2003) is the main route taken by nectar and this certainly appears to be the case for *Maxillaria coccinea* (Stpiczyńska et al., 2004). However, other authors claim that nectar is mainly transported along the symplast especially in cases where cutinized layers present in the cell wall may hinder its flow (Fahn, 2000). Final modification of the pre-nectar occurs within the nectary cells and, in the case of *S. sanguineum* well-developed endoplasmic reticulum and plastids are probably involved in this process. During the secretory stage, numerous vesicles are visible in the parietal cytoplasm and, since some of these appear to fuse with the plasmalemma, it is likely that a granulocrine mode of secretion operates here. Granulocrine secretion has also been observed within the nectary cells of *Hexisea imbricata* (Stpiczyńska et al., 2005).

The nectary cells of *S. sanguineum* have an organelle complement consistent with that found in secretory cells. They have conspicuous nuclei and contain dense, granular cytoplasm with small vacuoles, numerous mitochondria, ER, secretory vesicles and leucoplasts (Fahn, 2000). Some plastids of *S. sanguineum,* and *H. imbricata,* contain starch grains, which is absent in *M. coccinea*. The nectary of *S. sanguineum* has a vascular supply and sugars translocated in the sieve tubes accumulate in the nectary where they are stored as starch within amyloplasts. Starch is very common in the nectary cells of orchids (Figueiredo and Pais, 1992; Pais and Figueiredo, 1994; Stpiczyńska, 1997; Galetto et al., 1997) as well as other plants (Pacini et al., 2003) and can be used both as a source of nectar and energy for highly metabolic processes. However, in a number of orchid species, throughout the whole process of nectar secretion starch is absent from the nectary plastids and instead, they contain numerous lipid droplets (Stpiczyńska and Matusiewicz, 2001; Stpiczyńska et al., 2004). Frequently, the leucoplasts of these orchids, as well as those of *S. sanguineum*, become associated with the endoplasmic reticulum. The secretory cells of *S. sanguineum* usually contain SER which participate in the synthesis and transport of lipids (Fahn, 2000) as indicated by the numerous lipid droplets found within the cytoplasm of *S. sanguineum* and *H. imbricata* (Stpiczyńska et al., 2005). However, the reason for this dramatic increase in lipid production by the nectary cells of certain orchid species requires further study.

In conclusion, although the nectary of *S. sanguineum* shares many features with other ornithophilous species, it nonetheless differs from these in a number of important ways. These include its position upon the flower, the presence of microchannels within the cuticle as well as the dissolution of the middle lamella and the subsequent separation of epidermal cells. The differences mentioned above that contribute pollinator selection, confirm the hypothesis that ornithophily may have evolved along a number of separate routes (van der Cingel, 2001).

Struktura nektarników *Symphyglossum sanguineum* (Rchb.f.) Schltr. (Orchidaceae)

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Streszczenie

Ornitofilia występuje u wielu Orchidaceae, lecz nektarniki storczyków zapylanych przez ptaki rzadko są przedmiotem badań. Celem niniejszej pracy było zbadanie struktury nektarników *Symphyglossum sanguineum*, gatunku przypuszczalnie zapylanego przez kolibry. Nektarnik w kwiatach *S. sanguineum* znajduje się na wolnych krawędziach auriculi, które są wytworem warżki. Auricule tworzą kanał, przez który przesącza się nektar na powierzchnię labellum. Nektarnik zbudowany jest z jednowarstwowej epidermy i 2-3 warstw komórek subepidermalnych. Jest on zaopatrywany przez kolateralne wiązki przewodzące. Komórki nektarnika *S. sanguineum* mają grube celulozowe ściany komórkowe. Charakterystyczną cechą nektarnika jest rozpuszczenie blaszek środkowych w ścianach niektórych komórek epidermy wydzielniczej, co powoduje ich rozsunięcie. Przypuszczalnie proces ten ułatwia przesączanie się nektaru na powierzchnię nektarnika. Kutykula pokrywająca komórki epidermy wydzielniczej ma mikrokanaliki, lecz w przeciwieństwie do komórek nektarników innych dotąd badanych ornitofilnych storczyków, nie pęka ani też nie tworzy uwypukleń pod wpływem wydzielanego nektaru. Cytoplazma komórek wydzielniczych zawiera liczne mitochondria, krople lipidowe i gładkie retikulum endoplazmatyczne (SER) wypełnione osmofilnym materiałem. Niektóre plastydy w komórkach nektarnika mają nieliczne tylakoidy i pęcherzyki oraz osmofilne globule, podczas gdy w innych plastydach znajdują się ziarna skrobi. Błony SER są w kontakcie z plastydami, a osmofilny materiał zawarty w SER przypomina ciemno wybarwione globule w plastydach. Liczne pęcherzyki wydzielnicze w sąsiedztwie ściany komórkowej wskazują na możliwość sekrecji pęcherzykowej.