THE STRUCTURE OF NECTARY OF *PLATANTHERA BIFOLIA* L. *ORCHIDACEAE*

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SUMMARY

The anatomy and ultrastructure of floral nectary of *Platanthera bifolia* were studied. The epidermis inside the nectary spur showed characteristic features of secretory tissue. Many cells of this epidermis were protruded forming unicellular hairs. The protoplasts of secretory cells were characterized by few small vacuoles, a lot of mitochondria and leucoplasts, which stored starch before secretion. Numerous vesicles budded off from the endoplasmic reticulum and the Golgi apparatus were accumulated near plasmalemma and fused with it. This fact probably indicates that these structures are involved in secretory processes. Nectar was released onto the surface through the pores in a ruptured cuticle, which covered the walls of secretory hairs.

KEY WORDS: *Platanthera bifolia, Orchidaceae*, ruptured cuticle, nectary.

INTRODUCTION

The orchids are a special group of plants in many ways. Especially interesting is their perfect adaptation to insect pollination. A very important factor in attracting the pollinator is the nectar produced in floral nectaries. In many orchids there are also so-called extra-floral nectaries, which are associated with inflorescences and flowers but not directly involved in the pollination process. Such glands are present at the outer base of the sepals or on the surface of floral bracts as in *Cymbidium, Vanda, Oncidium* and many others (Elias 1983).

Many authors have reported upon different sizes, shapes and anatomical structures of orchids nectaries as well as various nectar composition (Baskin and Bliss 1969, Jeffrey et al. 1970, Faegri and van der Pijl 1971, Proctor and Yeo 1975, Pais et al. 1986). In spite of this diversity, most nectaries consist of small, tightly packed glandular cells with dense cytoplasm plenteous in the membranes of the endoplasmic reticulum and the Golgi apparatus and rich with ribosomes and mitochondries. On account of relatively large nucleus and sometimes reduced vacuoles, they resemble meristematic cells (Durkee 1983). The views on the manner of secretion by nectaries are based mainly on the studies of ultrastructural changes which occur during their development. Nevertheless, some authors have presented physiological and cytochemical data incorporated with quantative structural informations (Robards and Oates 1986, Terry and Robards 1987, Robards and Stark 1988, Sawidis 1991, Zellnig et al.1991).

The purpose of the undertaken research was the investigation of anatomy and ultrastructure of floral nectary of *Platanthera bifolia* L., a species inhabiting light forests and meadows, pollinated by nocturnal Lepidoptera.

MATERIAL AND METHODS

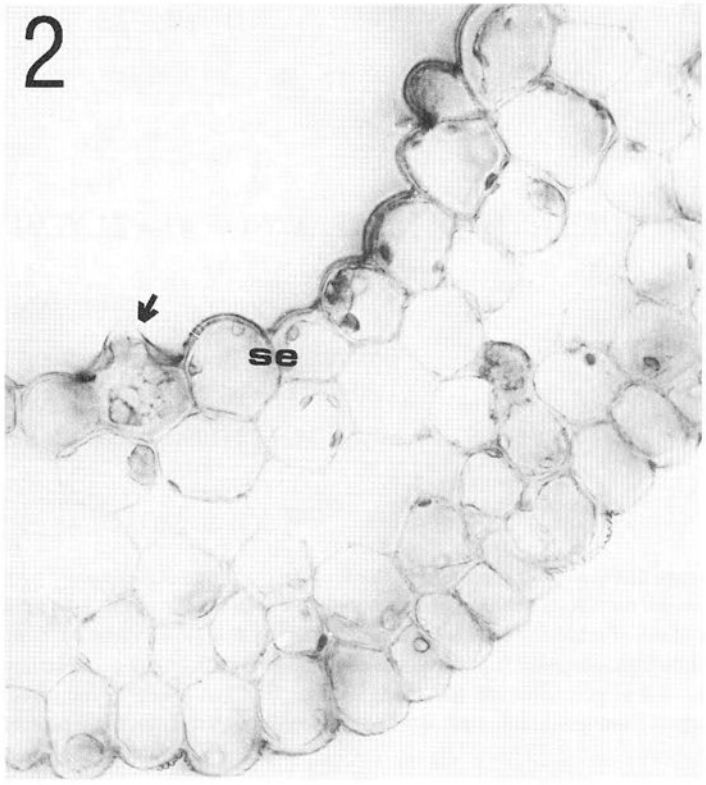
Flowers of *Platanthera bifolia* were collected between 15th and 20th of June 1994 and 1995, in a natural stand – forest near Lublin.

The structure of spur nectaries was investigated in three developmental stages: buds before nectar appeared in spur, freshly opened flowers with one third spur filled with nectar and flowers after anthesis, still with nectar in spur. Dissected nectaries were cut with a razor blade into fragments about 1 mm long and fixed in buffered 3% glutaraldehyde with paraformaldehyde, at room temperature, for 5h (Karnovsky 1965). After several rinses in phosphate buffer (pH 7,0) nectaries were postfixed in 2% osmium tetroxide for 2h at 0°C. Dehydrated material was embedded in epoxy resin (Spurr 1969). Ultrathin sections were stained with uranyl acetate followed by lead citrate (Reynolds 1963) and observed in electron microscope TESLA-340 at 60 kV.

Semithin sections about 1 μm were stained with 0,05% toluidine blue 0 for light microscope. Some sections from the fresh material were cut by hand and treated with JKJ and Sudan III for presence of starch and lipids or cutinized cell walls respectively.

RESULTS

The spur-shaped nectaries of the lesser butterfly orchid (*P. bifolia*) were formed from fragment of a labellum (Fig. 1). The cells of abaxial epidermis which covered the nectary spur were strongly vacuolized. Thin cuticle with striated surface was found on their external walls. Underneath three layers of



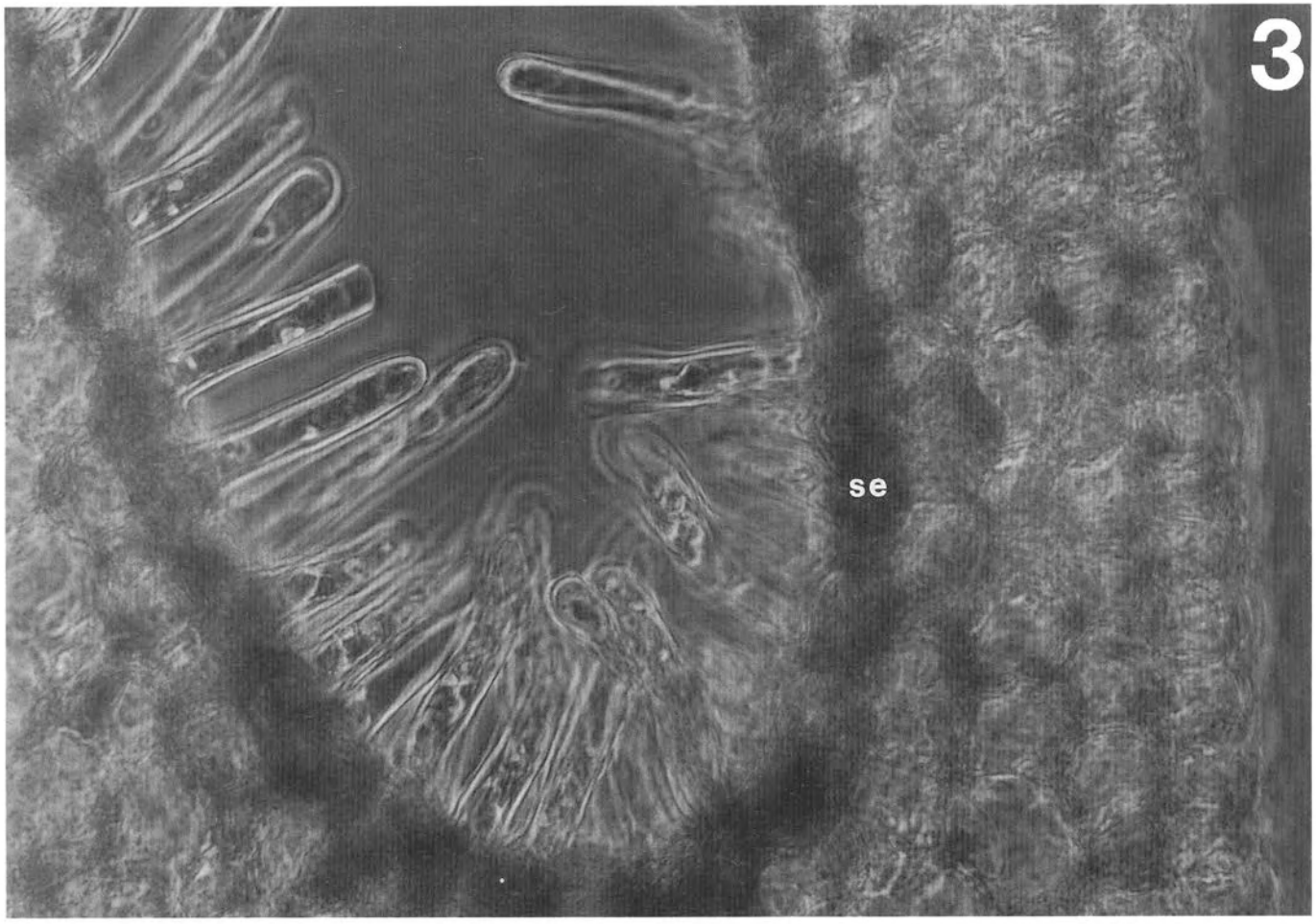


Fig. 1. The habit of *Platanthera bifolia* plant. Nectary spur is marked by arrow.
Fig. 2. The crossection of young nectary spur. The cell forming protrusion is marked by arrow, x350.
Fig. 3. The crossection of nectary spur in secretory stage. Secretory epidermis is distinctly darker than parenchyma cells and external epidermis. Inside the nectar cavity numerous hairs occur. LM, x500.
Abbreviations: se – secretory epidermis.

thin walled parenchyma cells appeared, with parietaly localized cytoplasm and usually one, large vacuole. In parenchyma two large vascular bundles occured. They consisted of phloem and xylem elements which were not branching beneath secretory tissue. The inner (adaxial) epidermis was composed of small cells with dense cytoplasm. Significant feature of these cells was creating of numerous protrudes which developed into the hairs which covered the inner surface of the spur (Figs 2, 3). There were, on the average, 190 hairs on 1 mm^2. The shorter ones were found near the entry of the spur, whereas the longest (about 200 μm in length) – in the central and terminal part. The small protrudes were observed in buds just before nectar appeared in the spur. Positive reaction for starch was detected in the inner epidermis of presecretory stage and in the parenchyma, especially around vascular bundles. In the later stages starch was not ascertained.

The parenchyma and the outer epidermis cells contained typical chloroplasts, whereas all cells of the inner, secretory epidermis were characterized by performance of spherical, agranal plastids of 3,5 μm in diameter, tightly surrounded by tubules of the endoplasmic reticulum. TEM observations revealed the existence of very dense stroma with scarce, peripherally located tubules and some plastoglobuli in these plastids. In the stage of buds they contained some small starch grains (Fig. 4) degradated when secretion started (Figs 5, 7). In the older nectaries plastoglobuli were larger and tubules formed structures resembling a prolamellar body (Fig. 6). The microbodies, with central, distinct crystaloid and lighter, cortical part were frequently observed in close vicinity of plastids (Fig. 8). Numerous ribosomes, wide cisterns of the endoplasmic reticulum and dictyosomes with disconnected vesicles belonged to the structures commonly occured in cytoplasm of secretory stage, especially beneath the plasmalemma. Fusion of these structures was often observed (Figs 9, 10). Mitochondria, sometimes elongated in shape and with dense tubules were concentrated near the cell wall (Fig. 9). Some lipid droplets were seen in cytoplasm of secretory cells both in freshly opened as well as in older flowers. Nucleus was located at the base of epidermal cell, and was never observed in the hair area. Flat cells of the secretory epidermis possesed usually one, electron empty vacuole, whereas the cells with protrusion and hairs had only few small vacuoles with dark, flocculent precipitations (Fig. 10). However, vacuolisation increased with the age of nectaries. The contact between epidermis and deeply located parenchyma was facilitated by numerous plasmodesmata (Fig. 11).

Smooth, cellulose outer walls of secretory epidermis were covered by cuticle, which was composed of reticular and lamellar layer. In the activity stage the disjunction from the wall and the rupture of the cuticle were observed (Fig. 12), but in flat cells the cuticle remained intact.

DISCUSSION

Flowers of the lesser butterfly orchid (*P. bifolia*) possess a spur nectary. In anatomical structure nectaries of this species show a high degree of specialisation. The inner epidermis was distinctly different from adjacent tissues and formed numerous hairs, which enlarge significantly the secretory surface. Such specialized secretory epidermis was not observed by Figueiredo and Pais (1992) in *Limodorum* spur. The cells of epidermis and neighbouring parenchyma of *Platanthera* spur were interconnected by plasmodesmata, which indicated, that the passageway of prenectar to the secretory epidermis was via the symplast, as in many others nectaries (Fahn 1979, Gunning and Hughes 1976, Sawidis et al. 1987, Terry and Robards 1987, Robards and Stark 1988). Because in *Platanthera* distinct apoplastic barriers were not ascertained, the transport within cell walls also seems to be possible.

In the parenchyma of the spur two large vascular bundles occured. In many species starch, initially derived from phloem sugars, can serve as the main source of nectar (Durkee et al. 1981, Durkee 1983). Since the bundles were not branched beneath the secretory epidermis in *Platanthera,* the involvement of assimilates produced by chloroplasts in parenchyma cannot be excluded. The accumulation of starch in plastids was observed only in a presecretory stage. Similar to *Platanthera*, degradation of starch connected with the start of secretion was noted by Meyberg and Kirsten (1981), Durkee et al. (1981), Figueiredo and Pais (1992), Pais and Figueiredo (1994).

In the final stage of anthesis the plastids in the secretory epidermis were still globoid, with dense stroma and numerous plastoglobuli. They formed also regular structures resembling prolamellar bodies. In *Limodorum* and *Epipactis* the tubular reticulum in plastids contained an unknown, dense osmiophillic material (Pais and Figueiredo 1994). Different character of plastids was revealed in *Passiflora* nectaries, where Durkee et al. (1981) observed their desintegration after nectarying cessation.

The dense cytoplasm of secretory cells of *Platanthera* was abundant in dictyosomes, dilated cisterns and tubules of the endoplasmic reticulum. However, the involvment of the Golgi apparatus in nectar secretion is the matter of a certain controversy (Durkee 1983), the participation of the endoplasmic reticulum in this process is unquestionable. In *Abutilon* (Robards and Oates 1986, Robards and Stark 1988) and *Hibiscus* (Sawidis et al. 1989, Sawidis 1991) structural evidence confirmed by cytochemical and physiological investigations indicated, that prenectar is actively loaded into the endoplasmic reticulum, closely associated with plasmalemma. Accumulation within the endoplasmic reticulum increases hydrostatic pressure and leads to the opening of "spinctered junctions" of cisternal space of the endoplasmic reticulum to the outside of plasmalemma. In many others nectaries the endoplasmic reticulum was recorded also as the most conspicuous feature of secretory cells (Durkee 1983, Fahn 1988, Kronestedt-Robards and Robards 1991). Since in *Platanthera* numerous vesicles budded off from the endoplasmic reticulum and the Golgi apparatus, probably granulocrine process of secretion occured.

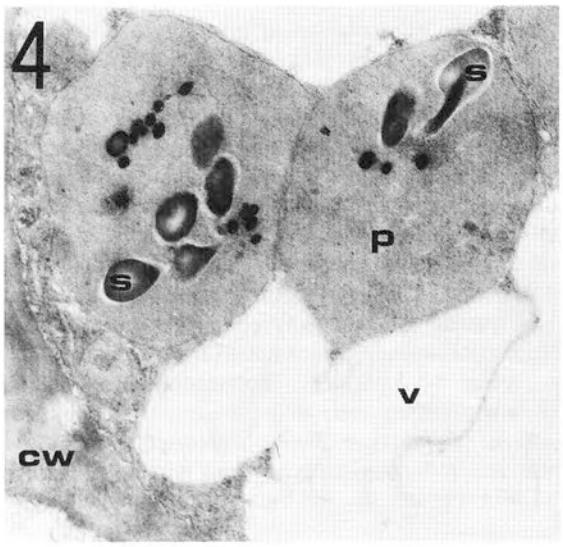
The secretory vesicles appeared near the plasmalemma and gathered particulary in those places, where the cuticle covering cell wall was ruptured, probably under the influence of the secreted nectar. Similar manner of exudation took place in *Pulmeria* (Mohan and Inamdar 1986) and *Limodorum* (Figueiredo and Pais 1992).

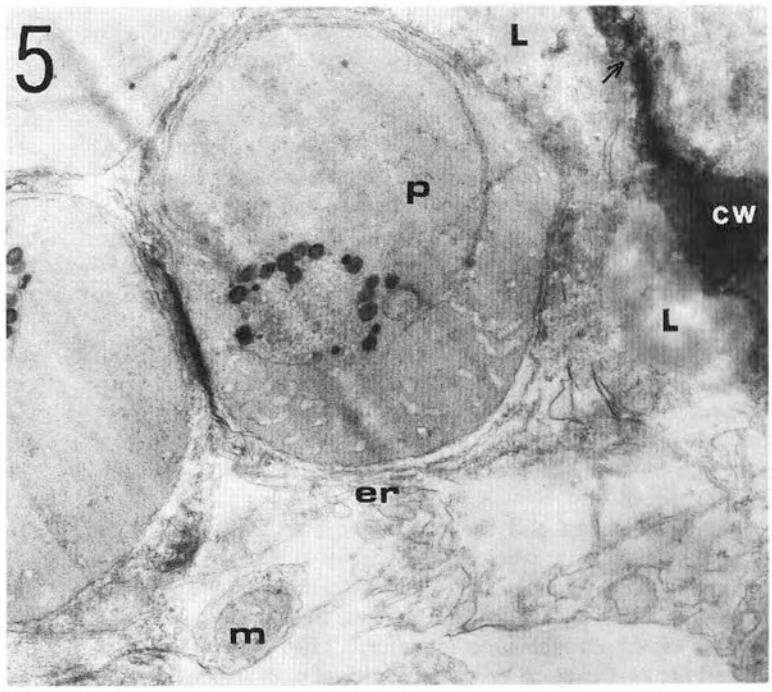
LITERATURE CITED

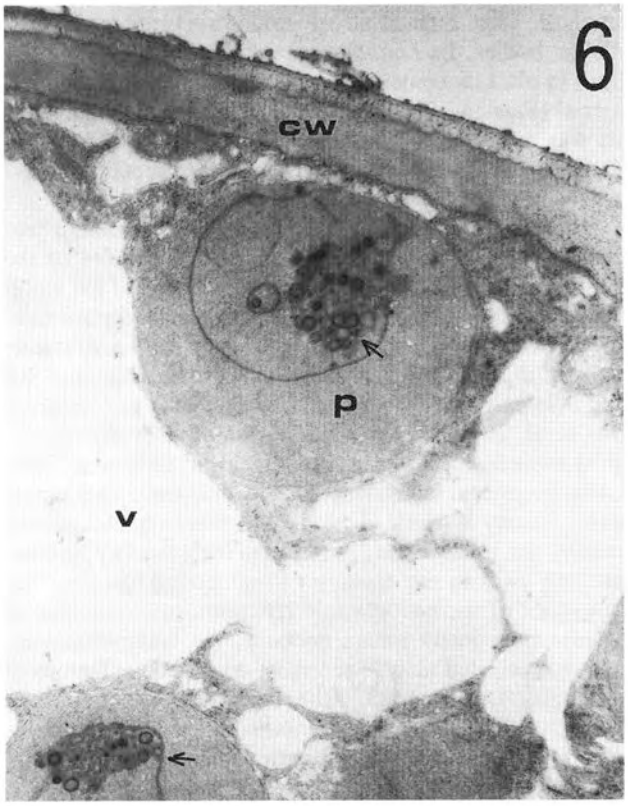
BASKIN J.S., BLISS C.A., 1969. Sugar occuring in the extrafloral exudates of the *Orchidaceae*. Phytochemistry 8: 1139-1145.

DURKEE L.T., GALL D.J., REISNER W.H., 1981. The floral and extrafloral nectaries of *Passiflora* I. The floral nectary. Amer. J. Bot. 68: 453-462.

DURKEE L., 1983. The ultrastructure of floral and extrafloral nectaries. In: The biology of nectaries. Bentley and Elias ed. Columbia university Press. New York.







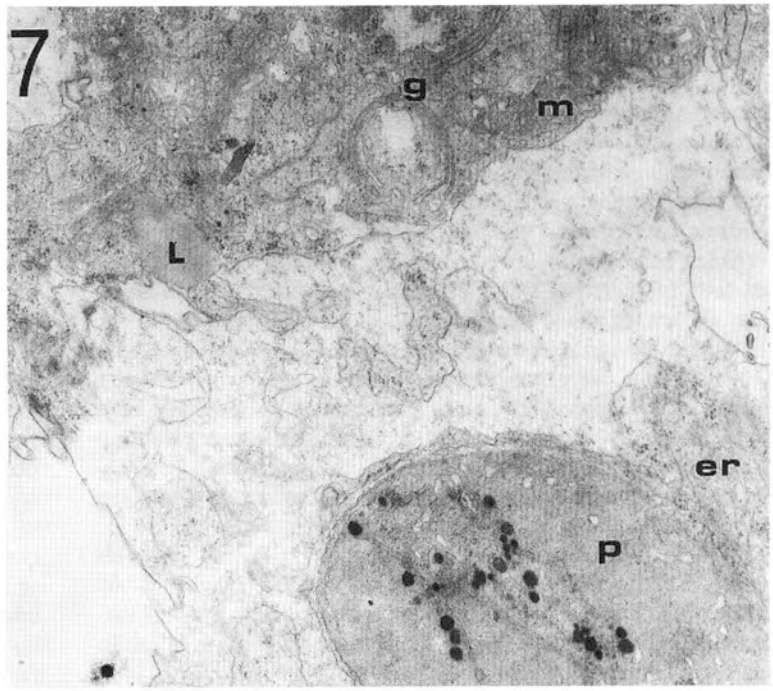
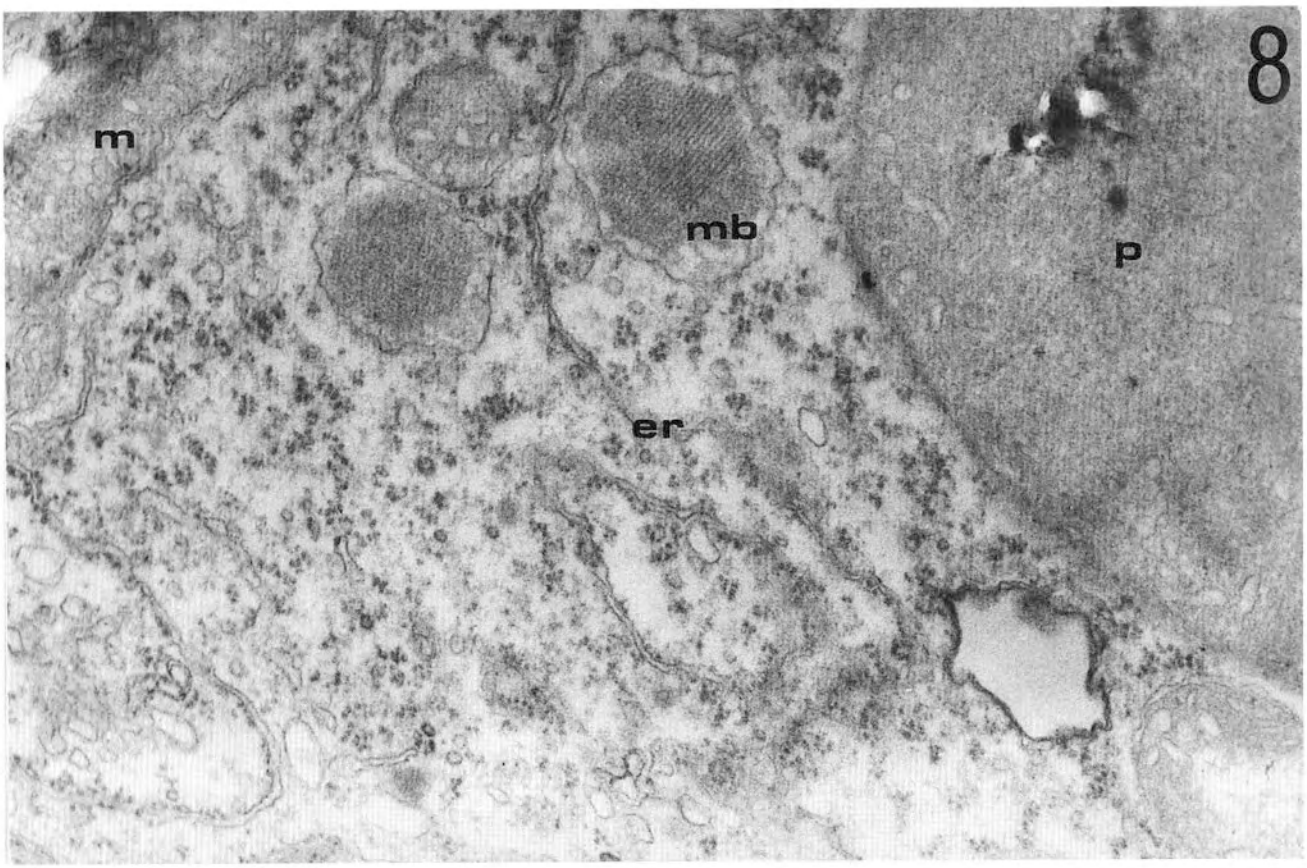


Fig. 4. Plastids in cells of inner epidermis of presecretory stage with starch grains and some plastoglobuli, x10000.
Fig. 5. Plastid from epidermis of secretory stage without starch and with scarce, peripherical tubules. In cytoplasm lipid droplets occur. Plasmodesmata are marked by the arrow, x16000.
Fig. 6. Plastids from epidermis of postanthesis stage with tubules and plastoglobuli forming structure resembling prolamellar body, x10000.
Fig. 7. Fragment of protoplast of secretory hair in activity stage. Cytoplasm packed with tubules of ER, dictyosomes, vesicles and mitochondria. Plastid with dispersed plastoglobuli, x14000.
Abbreviations: CW – cell wall, P – plastid, m – mitochondrion, er – endoplasmic reticulum, g – Golgi apparatus, L – lipid droplet, V – vacuole, s – starch



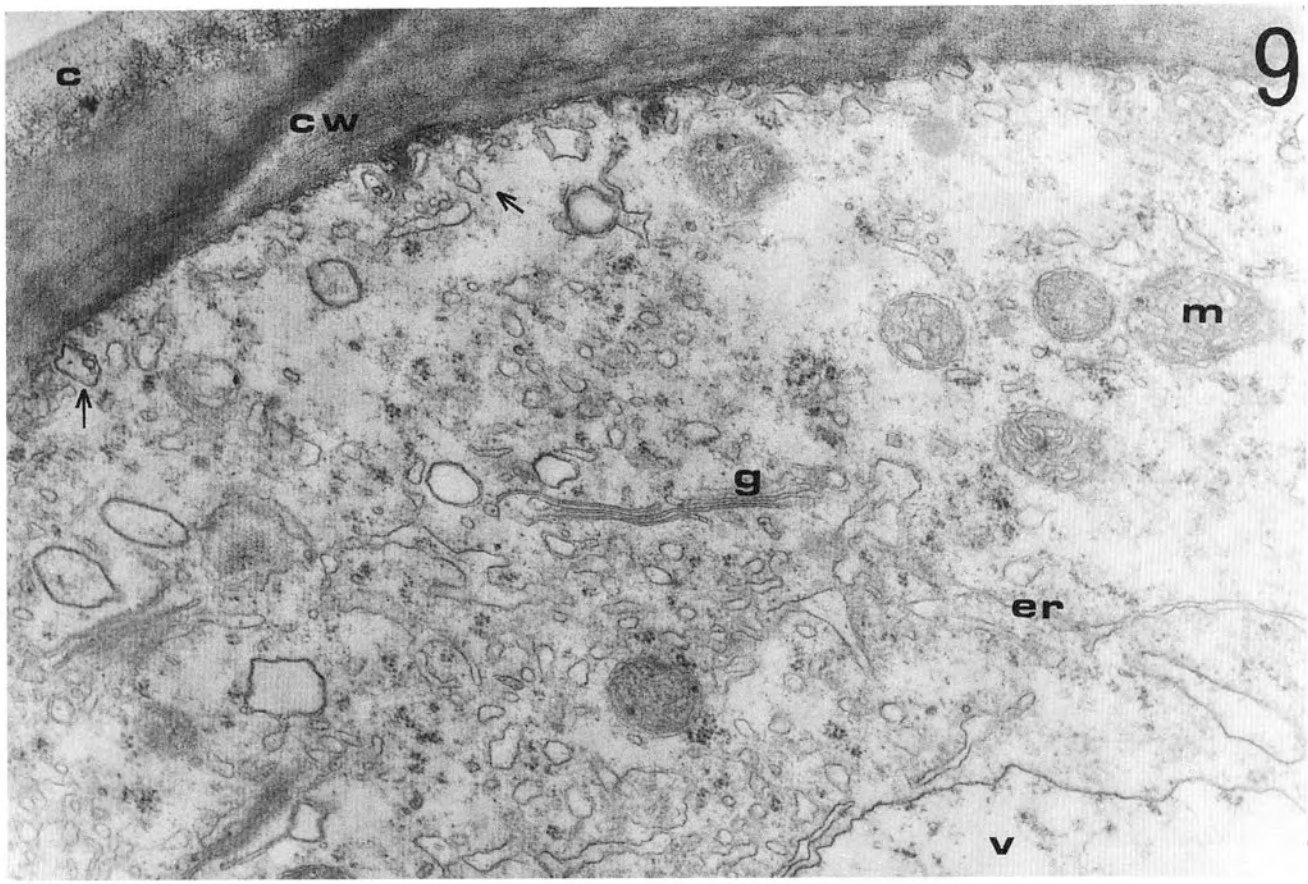
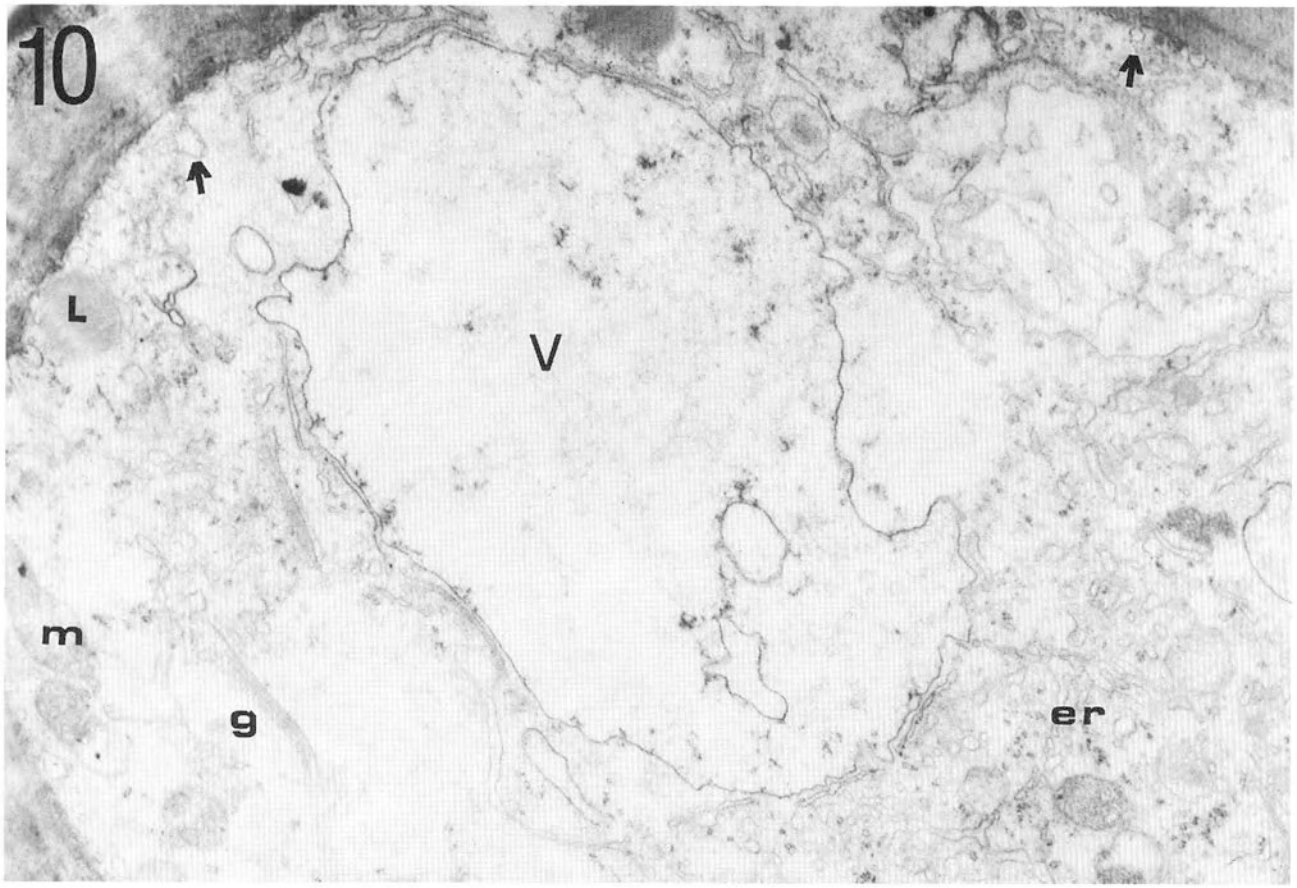
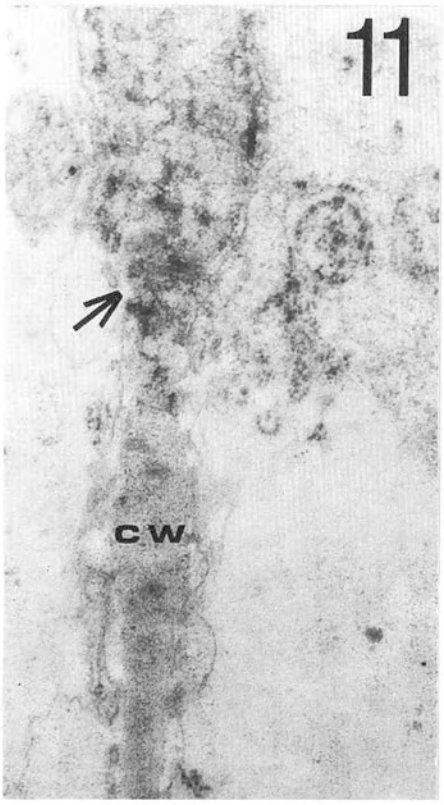


Fig. 8. Fragment of protoplast of secretory hair with numerous ribosomes and tubules of ER. Near plastid some microbodies occur, x46000.
Fig. 9. Numerous secretory vesicles (arrows) gathered near plasmalemma, some connected to it. Beneath cell wall many mitochondria, dictyosomes and membranes of ER occur, x23000.
Abbreviations: CW – cell wall, C – cuticle, P – plastid, m – mitochondrion, mb – microbodies, er – endoplasmic reticulum, g – Golgi apparatus, V – vacuole.





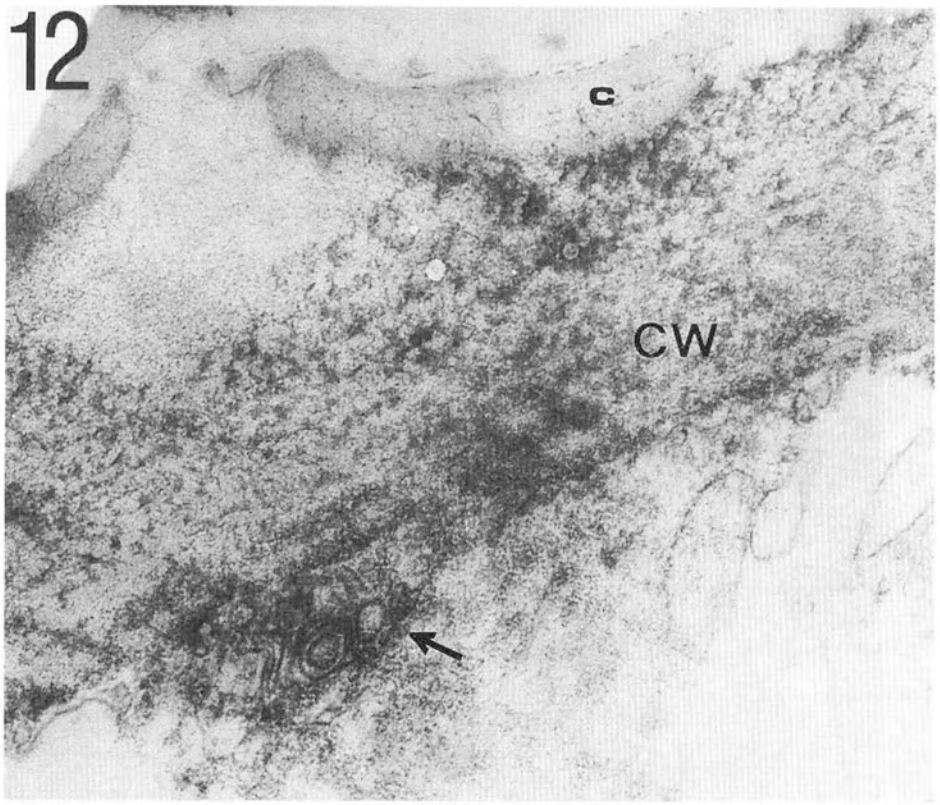


Fig. 10. Crossection of secretory hair with vacuole filled with flocculent percipitations, cytoplasm with many membranes of ER and dictyosomes. Near plasmalemma secretory vesicles (arrows) and lipid droplets occur, x10000.
Fig. 11. Fragment of cell wall in secretory epidermis. Plasmodesmata are marked by the arrow, x40000.
Fig. 12. Disconnected cuticle covered cell wall of secretory hair. Below secretory vesicles occur (arrow), x53000.
Abbreviations: CW – cell wall, C – cuticle, m – mitochondrion, er – endoplasmic reticulum, g – Golgi apparatus, L – lipid droplet, V – vacuole.

ELIAS T., 1983. The extrafloral nectaries. In: The biology of nectaries. Bentley and Elias ed. Columbia University Press. New York.

FAEGRI K. VAN DER PIJL L., 1971. The principles of pollination ecology. Pergamon Press. Oxford.

FAHN A., 1979. Ultrastructure of nectaries in relation to nectar secretion. Amer. J. Bot. 57: 977-985.

FAHN A., 1988. Secretory tissues in vascular plants. New Phytol. 108: 229-257.

FIGUEIREDO A.C., PAIS M.S., 1992. Ultrastructural aspect of the nectary spur of *Limodorum abortivum* (L.) Sw. *Orchidaceae*. Ann. Bot. 70: 325-331

GUNNING B.E.S., HUGHES I.E., 1976. Quantative assessment of symplastic transport of pre nectar into trichomes of *Abutilon* nectaries. Austr. J. Plant Physiol. 3: 619-637.

JEFFREY D.C., ARDITTI J., KOOPOVITZ H., 1970. Sugar content in floral and extrafloral exudates of Orchids: Pollination, myrmecology and chemotaxonomy implications. New Phytol. 69: 187-195.

KARNOVSKY M.J., 1965. A formaldehyde - glutaraldehyde fixative of high osmolarity for use in electron microscopy. J. Cell Biol. 27. 137A.

KRONESTEDT-ROBARDS E., ROBARDS A.W., 1991. Exocitosis in gland cells. In: Endocytosis, Exocytosis and Vesicle Traffic in Plants. Ed. by C.R. Hawes, J.O.D. Coleman, D.E. Evans. Cambridge Univ. Press.

MEYBERG M., KIRSTEN U., 1981. The nectaries of *Aptenia cordifolia* - ultrastructure, translocation of ^{14}C-labelled sugars and possible pathway of secretion. Z. Pflanzenphysiol. 104: 139-147.

MOHAN M., INAMDAR I., 1986. Ultrastructure of extrafloral nectaries of *Pulmeria rubra* L. Ann. Bot. 57: 389-401.

PAIS M.S., CHAVES DAS NEVES H. J., VASCONCELOS A.M., 1986. Aminoacid and sugar content of the nectar exudate from *Limodorum abortivum* (*Orchidaceae*). Comparison with *Epipactis atropurpurea* nectar composition. Apidologie 17: 125-136.

PAIS M.S., FIGUEIREDO A.C., 1994. Floral nectaries from *Limodorum abortivum* (L.) Sw. and *Epipactis atropurpurea* Rafin. (*Orchidaceae*): ultrastructural changes in plastids during the secretory process. Apidologie 25: 615-626.

PROCTOR M., YEO P., 1975. The pollination of flowers. Collins st. James Place ed., London. pp 230-255.

REYNOLDS R. S., 1963. The use of lead citrate at high pH as electronopaque stain in electron microscopy. J. Cell Biol. 17: 208-213.

ROBARDS A.W., OATES K., 1986. X-ray microanalysis of ion distribution in *Abutilon* nectary hairs. J. Exp. Bot. 39: 573-586.

ROBARDS A.W., STARK M., 1988. Nectar secretion in *Abutilon*: a new model. Protoplasma 142: 79-91.

SAWIDIS T., ELEFTHEIROU E.P., TSEKOS I., 1987. The floral nectaries of *Hibiscus rosa-sinensis* L. II. Plasmodesmatal frequences. Phyton 27: 155-164.

SAWIDIS T. ELEFTHEIROU E.P., TSEKOS I., 1989. The floral nectaries of *Hibiscus rosa-sinensis* L. III. A morphometric and ultrastructural approach. Nord. J. Bot. 9: 63-71.

SAWIDIS T., 1991. A histochemical study of nectaries of *Hibiscus rosa-sinensis*. J. Exp. Bot. 42: 1477-1487.

SPURR A.R., 1969. A low viscosity epoxy resin embedding medium for electron microscopy. J. Ultrastruct. Res. 26: 31.

TERRY B.R., ROBARDS A.W., 1987. Hydrodynamic radius alone governs the mobility of molecules through plasmodesmata. Planta 171: 145-157

ZELLNIG G., KRONESTEDT-ROBARDS E.C., ROBARDS A.W., 1991. Intercellular permeability in *Abutilon* nectary trichomes. Protoplasma 161: 150-159.

BUDOWA NEKTARNIKA PODKOLANA BIAŁEGO *PLATANTHERA BIFOLIA* L. *ORCHIDACEAE.*

STRESZCZENIE

Przedmiotem badań była anatomia i ultrastruktura nektarnika kwiatowego podkolana białego (*Platanthera bifolia* L.). Nektar gromadzony był w ostrodze, wewnątrz której epiderma miała charakter tkanki wydzielniczej. Wiele komórek epidermy tworzyło uwypuklenia do wnętrza w postaci włosków. Protoplasty ich charakteryzowały się obecnością kilku niewielkich wakuoli, licznymi mitochondriami oraz leukoplastami, które przed sekrecją zawierały skrobię. Wiele pęcherzyków odrywających się od retikulum endoplazmatycznego i aparatów Golgiego gromadziło się w sąsiedztwie plazmolemy. Wielokrotnie obserwowano również fuzję tych struktur, co wskazuje na ich możliwe zaangażowanie w procesie wydzielniczym. Nektar uwalniany był na zewnątrz poprzez pęknięcia kutykuli pokrywającej ściany komórek włosków.

SŁOWA KLUCZOWE: *Platanthera bifolia* L., *Orichdaceae,* pęknięcia kutykuli, nektarnik.