

OSMOPHORES OF THE FRAGRANT ORCHID *GYMNADENIA CONOPSEA* L. (ORCHIDACEAE)

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ABSTRACT

Osmophores of *Gymnadenia conopsea* are located on the adaxial surface of labellum and on distal parts of two lateral sepals. Osmophore cells are characterised with a large nucleus, and dense, granular cytoplasm, which contains numerous membranes of ER and large lipid droplets. Plastids are probably involved in the synthesis of fragrant substances and, contrary to the most of investigated orchid species, they do not contain starch. Numerous secretory vesicles take part in the secretion. Secreted fragrant substance migrates across the cell wall and through the pores in the cuticle. It is not accumulated on the osmophore cell surface.

KEY WORDS: *Gymnadenia conopsea* L., Orchidaceae, osmophore, anatomy, ultrastructure.

INTRODUCTION

Fragrant substances produced in osmophores of many orchids serve as attractants for pollinators. The specificity of the scent is fairly significant and creates a precise isolating mechanism between closely related species, depending on limited quantity or even one, most efficient species of pollinator (Williams, Dodson 1972; Nillson 1985). The fragrance is very important for these flowers, which are recognised and visited by nocturnal moths (*Platanthera*, *Angraecum*, *Brassavola*, *Epidendrum*, *Habenaria*) and for majority of nectarless orchids pollinated by Euglossine bees, where the scent is the only attracting factor as *Gongora*, *Coryanthes*, *Pescatoria*, *Stanhopea*, *Catasetum* and many others (Dodson et al. 1969; Williams, Dodson 1972; Williams, Whitten 1983).

The scent of melitophilous and psychophilous or phalaenophilous flowers is pleasant and it contains mostly different terpenoids and/or aromatic compounds (Nillson 1985, Williams, Whitten 1993). The myophilous flowers, such as *Bulbophyllum distans*, *Disa lugens* emit in turn an unpleasant, urinous or aminos smell (trimethylamine) – as in the case of *Restrepia antennifera*, *Cirrhopetalum omatissimum*. Butyric acid is present in the smell of flowers of *Pleurothallis cillata*, *P. fuegi*, *Scaphosepalum* and capronic acid – in *Himantoglossum hircinum* (Vogel 1990).

Osmophores are located on the adaxial surface of flowers and frequently occur on particularly modified perianth fragments. Sometimes osmophores are not morphologically altered, in other cases they are distinguishable by different colour of flowers in this place (Stern et al. 1987; Vogel 1990).

The evidence of the presence of osmophore tissue is provided by the selective staining with neutral red or/and with Sudan

black B (Stern, Curry 1986; Vogel 1990). The osmophore tissues are characterized also by negative electric potential in comparison to the neighbouring parenchyma and storing the reserve materials in subsecretory tissue, used rapidly during the emission stage. Because of cytotoxicity, the secreted substances are not accumulated in large quantities but they are produced and emitted periodically. Temporarily, the synthesis of fragrance is adapted to the activity of visitors and may differ in pollinated and unpollinated flowers (Vogel 1990).

The knowledge on the structure of osmophores of some neotropical orchid species is detailed (Pridgeon, Stern 1983; Stern, Curry 1986; Stern et al. 1987; Curry 1987; Curry et al. 1988), but the studies on fragrance producing glands in European orchids are fragmentary or absent.

The purpose of this paper was to examine the osmophore structure in flowers of *Gymnadenia conopsea* L.

MATERIAL AND METHODS

Gymnadenia conopsea plants were collected from a natural stand near Chełm. The studies were conducted on the flowers representing the stage of 3 mm – long bud without scent, and the first and fourth day of anthesis with intensive scent resembling lilac fragrance.

Preliminarily, osmophores were identified by submerging the flowers in 0.01% neutral red bath for 2 h and 24 h. After washing in tap water, the material was sectioned and examined with LM. Dissected sepals were also placed separately in the closed, small glass dishes to ascertain which of them emit a fragrance.

Samples for light (LM) and an electron microscope (TEM and SEM) were prepared according to the procedure described in

earlier paper on nectaries (Stpiczyńska 1997), with the exception of one part of material for SEM which was dried at the critical point of CO₂, coated with gold and examined with a TESLA microscope at the voltage 25 kV.

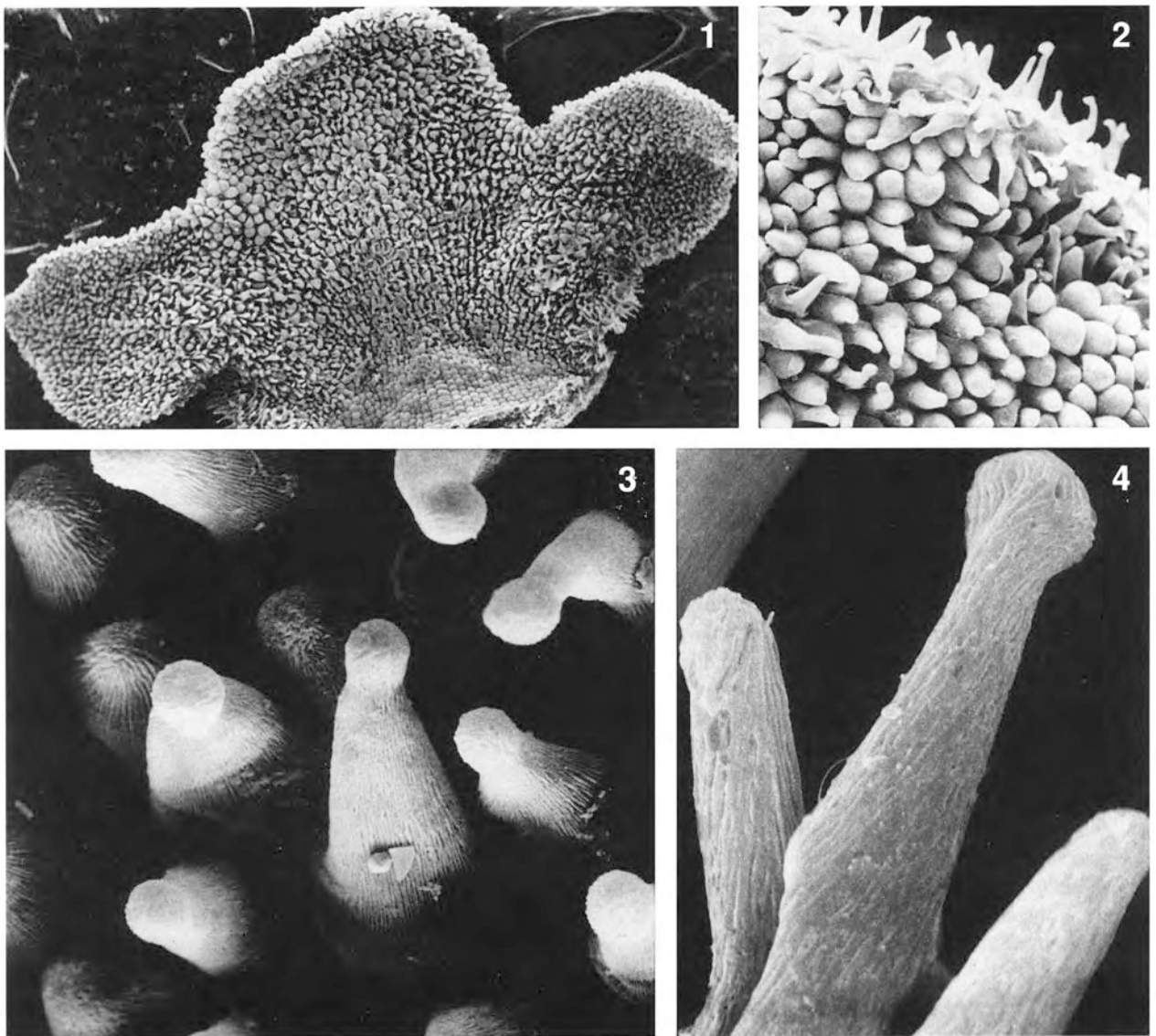
RESULTS

The SEM observations revealed, that osmophore was covered by cone-shaped epidermal cells, much longer on the margins of labellum, where they took shape of unicellular trichomes, usually bulbous at the end (Figs 1, 2). The cuticle covering the cells was ridged on the whole surface (Figs 3, 4). In the upper part of secretory cells (in cone-shaped as well as in trichomes) numerous pores appeared in cuticle (Figs 5, 6). They were absent in the presecretory stage. Stomata occurred in the abaxial epidermis of all sepals, except the labellum.

Staining live tissue with neutral red revealed an intensive accumulation of the stain in the vacuoles of the cells of adaxial

surface of labellum, particularly in its marginal parts (Figs 7, 8) and poorly, in comparison with labellum, in the distal parts of two lateral sepals. The result of the staining was already visible after 2 h and it did not change during 24-hour bath in neutral red. Stained parts of the perianth emitted also the most intensive odour. The differences in the intensity of secreted scent throughout a day were not distinguishable by olfaction. In LM the secretory cells were distinguished from deeply located parenchyma by dense cytoplasm and large nucleus (16 µm diameter in comparison with 9 µm in parenchyma cells). Little vacuoles, a few in the bud stage, increased after anthesis. During high secretory activity the cytoplasm contained also many lipid droplets, much larger in older flowers (Fig. 9). Plastids were observed exclusively in epidermal cells and they did not contain starch at any stage. External walls of epidermal cells were about twice as thick as the remaining ones. Numerous vascular bundles run in parenchyma.

TEM observations showed frequent occurrence of plasmodesmata between the secretory cells. Their cytoplasm was rich in



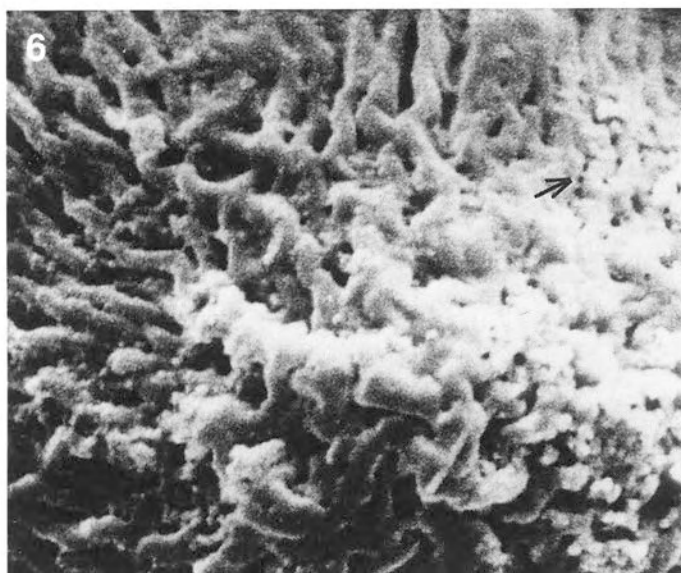
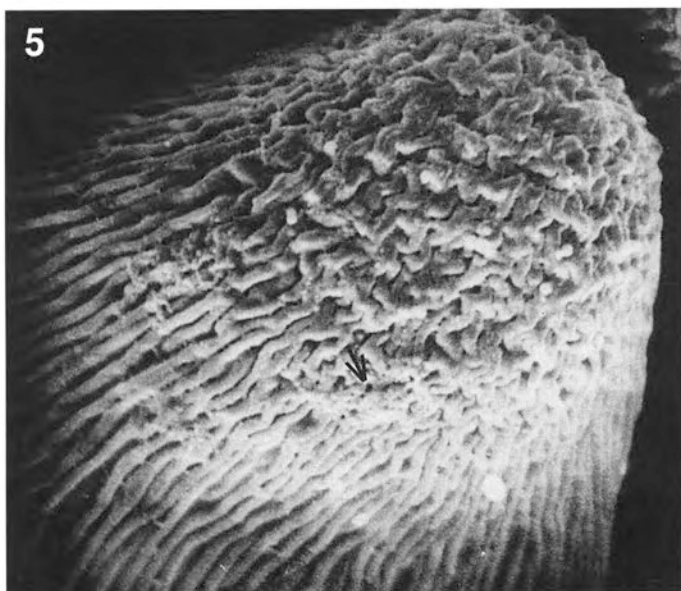
Figs 1-4. Osmophore morphology, SEM.

Fig. 1. Adaxial surface of *G. conopsea* labellum, × 60.

Fig. 2. Margin of the labellum with cone-shaped and trichomous cells, × 220.

Fig. 3. Cone-shaped cells covered with ridged cuticle, × 1170.

Fig. 4. Trichomous cells from the margin of labellum with wider uppermost part, × 3100.

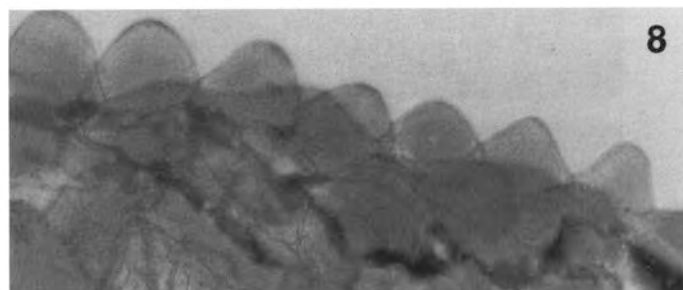
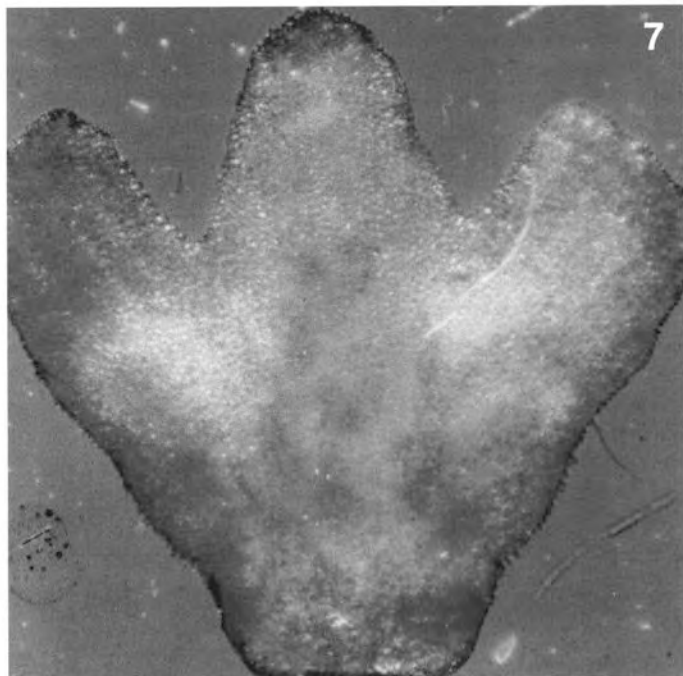


Figs 5-6. Surface of osmophore cells, SEM.

Fig. 5. Cone-shaped cell in secretion stage with pores between cuticle ridges (arrow), $\times 6000$.

Fig. 6. Magnified fragment of the top of cell surface from Fig. 5 with numerous pores in cuticle (arrow), $\times 8000$.

ribosomes, tubules of RER and wide cisterns of SER at the activity stage (Figs 10, 11). Membranes of SER were in close connection with plastids (Figs 12, 13). In osmophore cells the characteristic feature of plastids were tubules with osmiophilic content. At the bud stage, plastids had an oval shape and at the activity stage, they assumed ameboid forms. In the vicinity of plastids, mitochondria and lipid droplets, globoid or irregular in shape, appeared. They were not connected with ER membranes (Fig. 11). Numerous secretory vesicles were present in cytoplasm, especially near plasmalemma (Fig. 13). Moreover, at the activity stage many of vesicles gathered in deep membrane invaginations just before their content being released outside of protoplast (Figs 14, 15, 16). The content of vesicles was very similar in appearance to the inside of intraplastidal tubules (Figs 12, 13). The migration of the secreted substances across the cell wall and cuticle was frequently noted (Fig. 17), however, secreted substance was not accumulated on the surface of osmophore cells.



Figs 7-9. Cross section of the osmophore, LM.

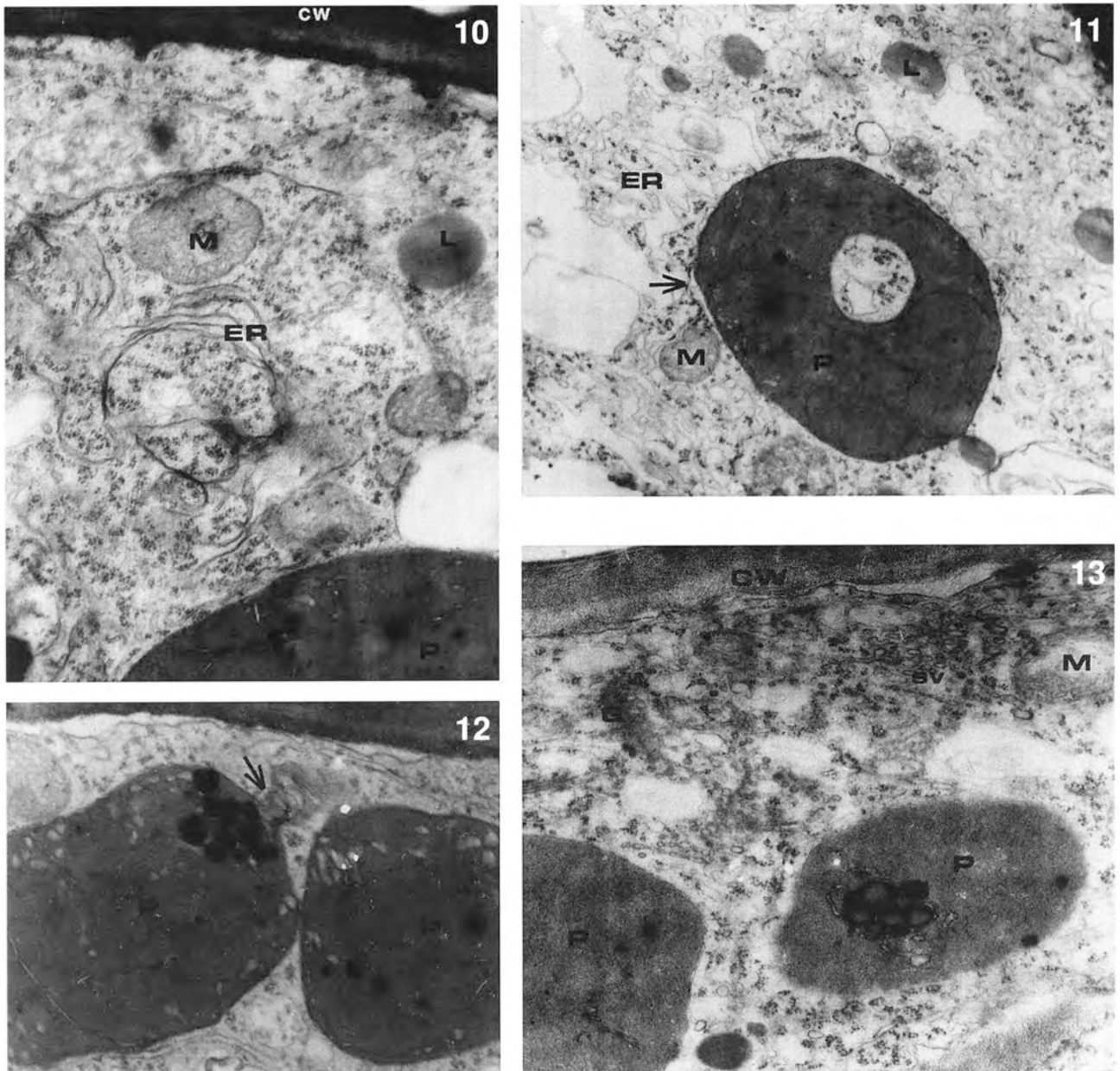
Fig. 7. Accumulation of stain on the margins of labellum after treatment with neutral red, $\times 15$.

Fig. 8. Vacuoles of osmophore cells stained with neutral red, $\times 417$.

Fig. 9. Cells from the margin of labellum with large lipid droplets in cytoplasm (arrows), $\times 500$.

DISCUSSION

In *G. conopsea*, the floral parts, which bear osmophore tissue are not especially modified, similarly as in *Platanthera* (Vogel 1990). Many characteristic features of fragrant orchid osmophores such as a large nucleus, a few small vacuoles, dense cytoplasm with numerous tubules of ER, mitochondria and lipid droplets were previously noted in other orchids (Vogel 1990; Stern et al. 1987; Curry et al. 1988; Stępczyńska 1993). Significant difference was the lack of starch in *G. conopsea*



Figs 10-13. Ultrastructure of osmophore cells at activity stage, TEM.

Fig. 10. Cytoplasm of trichome with numerous ribosomes, tubules of ER and lipid droplet, $\times 14\,000$.

Fig. 11. Plastid of ameboid shape in close contact with ER (arrow), $\times 14\,000$.

Fig. 12. Plastids with internal tubules containing osmiophilic globules and membranes in contact with ER (arrow), $\times 10\,000$.

Fig. 13. Plastids with large osmiophilic globules connected with intraplasmic membranes. Secretory vesicles near cell wall, $\times 10\,000$.

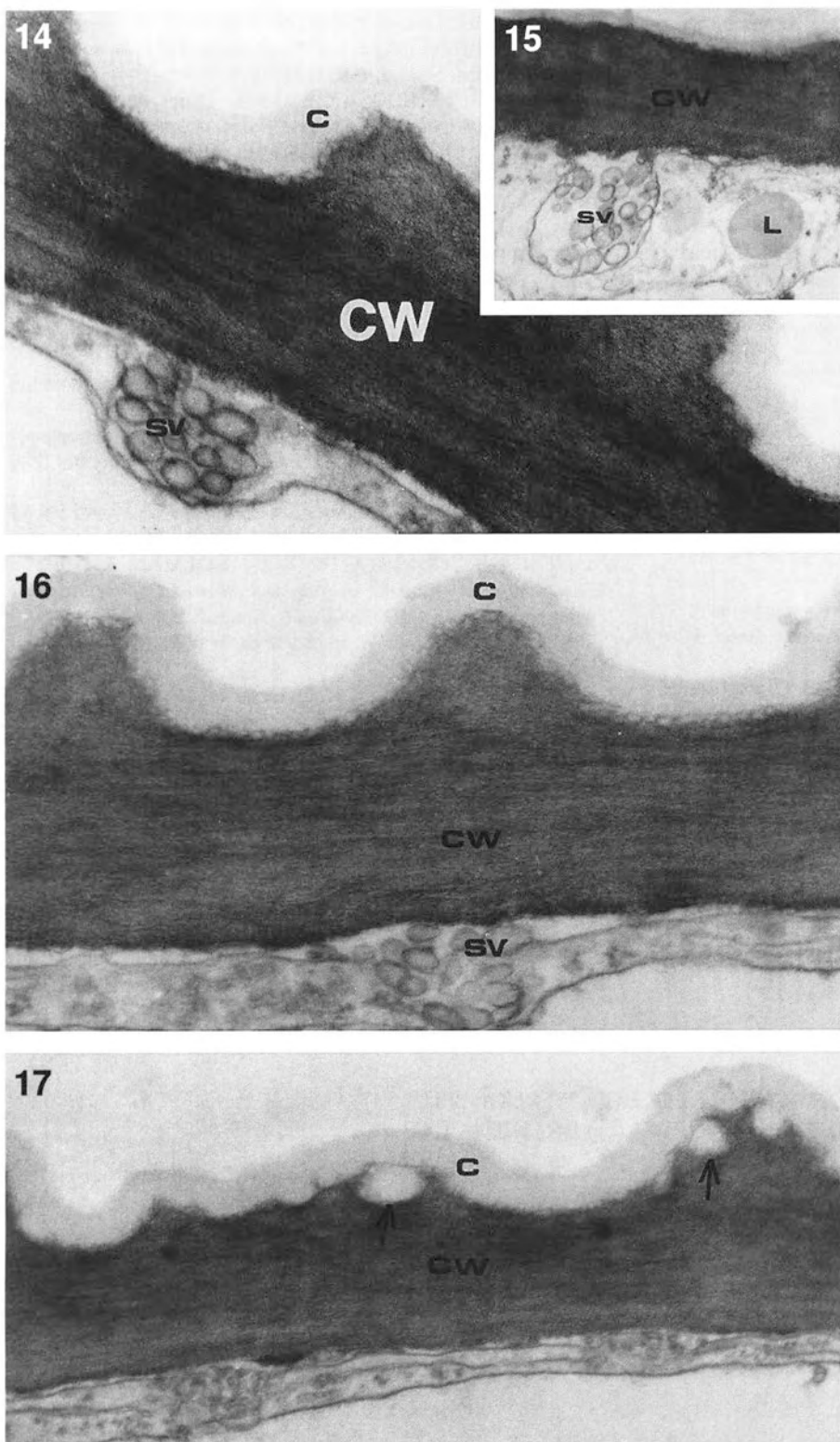
Abbreviations to Figs 10-17

CW – cell wall, C – cuticle, P – plastid, ER – endoplasmic reticulum, G – Golgi apparatus, M – mitochondrion, N – nucleus, V – vacuole, L – lipid droplet, SV – secretory vesicle.

plastids at any developmental stage. Starch was usually observed in the majority of osmophore plastids and, exceptionally, starchless plastids were reported by Swanson et al. (1980) in glandular ovarian trichomes of *Cypripedium*, which were responsible for production and emanation of lipid soluble odours. Remaining plastid features, such as electron dense stroma and osmiophilic globules connected with internal tubules, were typical for osmophore cells as well as for glandular trichomes of *Teucrium scorodonia* (Sevinat-Pinto, Antunes 1991), secretory cavities of *Citrus deliciosa* (Bosabalidis,

Tsekos 1982b), resin ducts of *Pinus* (Fahn 1988) or orchid nectaries (Pais, Figueiredo 1994; Stpiczyńska 1997).

In *G. conopsea*, numerous plastoglobuli were associated with intraplasmic membranes. Analogous structures were interpreted in *Stanhopea* osmophores and *Citrus* secretory cells as precursors for synthesis of fragrant material (Bosabalidis, Tsekos 1982a; Curry et al. 1988). The evident contact of plastids with tubules of the smooth endoplasmic reticulum might indicate the involvement of plastids in processes of synthesis of volatile substances. Bosabalidis and Tsekos (1982a) reported that the con-



Figs 14-17. Migration of secreted substances across the cell wall, TEM.

Fig. 14. Secretory vesicles inside of membrane invagination, $\times 20\,000$.

Fig. 15. Secretory vesicles and large lipid droplet in cytoplasm beneath cell wall, $\times 16\,000$.

Fig. 16. Secretory vesicles with content about to be released to the cell wall, $\times 20\,000$.

Fig. 17. Secreted material visible in cell wall, just beneath cuticle, (arrow), $\times 18\,000$.

nection of both organelles is related with the transport of secretory products from the plastids into the ER. Curry (1987) ascertains, that the SER is the place of transport or/and synthesis of terpenoids and the multiplicity of compartments for the mevalonic acid pathway (this author identified it also between the inner and the outer mitochondrial membranes) may be necessary for the synthesis of variety of different terpenoid final products.

In cytoplasm of *G. conopsea* cells, especially at the stage of activity, numerous lipid droplets, not connected with the ER

occurred. The lipids were present also in osmophores of many other orchid species, where they were regarded as physical counterparts of the fragrance production (Swanson et al. 1980; Pridgeon, Stern 1983; Curry et al. 1988).

As numerous vesicles beneath plasmalemma indicate, the release of fragrant substances in *G. conopsea* osmophores occurred probably due to granulocrine secretion. However, if the lipids present in the cytoplasm are also the compounds of the produced scent, they are secreted independently from the secretory vesicles. Stern et al. (1987) presumed that in

Stanhopea aromatics and terpenoids pass through plasmalemma as individual particles (eccrine secretion), whereas granulocrine model of secretion operates in *Restrepia* osmophores (Pridgeon, Stern 1983).

The fragrant substances in *G. conopsea* osmophores migrated through the cell wall and numerous pores in cuticle, similarly to *Restrepia* (Pridgeon, Stern 1983). However, the accumulation of secreted substance was not visible on the cell surface, even during live tissue observation in SEM, probably because of their rapid volatilization. The droplets of secreted substances were observed in *Stanhopea*, where they gathered in liquid form on the osmophore surface (Vogel 1990; Stern et al. 1987).

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OSMOFORY GÓŁKI DŁUGOOSTROGOWEJ *GYMNADENIA CONOPSEA* (L.) ORCHIDACEAE

STRESZCZENIE

Osmofory gółki długoostrogowej zajmowały doosiową powierzchnię warzki oraz końcowe odcinki dwóch bocznych listków okwiatu zewnętrznego okółka. Komórki wydzielnicze charakteryzowała obecność dużego jądra, gęstej, ziarnistej cytoplazmy zawierającej krople lipidowe oraz wielu błon retikulum endoplazmatycznego. Plastydy, które są prawdopodobnie zaangażowane w syntezę związków zapachowych, w przeciwieństwie do większości osmoforów nie zawierały skrobi. W sekcji uczestniczyły liczne pęcherzyki wydzielnicze. Wydzielina migrująca przez ścianę komórkową wydostawała się na powierzchnię przez pory w kutykuli.

SŁOWA KLUCZOWE: *Gymnadenia conopsea* L., Orchidaceae, osmofor, anatomia, ultrastruktura.