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THE STRUCTURE OF ELAIOPHORES IN *ONCIDIUM CHEIROPHORUM* RCHB.F. AND *ORNITHOCEPHALUS KRUEGERI* RCHB.F. (ORCHIDACEAE)

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S u m m a r y

The shining appearance of the flowers of *Oncidium cheirophorum and Ornithocephalus kruegeri* results from the presence of lipids on the flower surface. The lipids are produced by elaiophores – secretory structures situated symmetrically at the base of the labellum or upon the callus.

In *O. cheirophorum*, the elaiophores are epithelial type. They consist of one layer of cuboidal secretory cells and subsecretory parenchyma. The thick cuticle covering the outer, tangential wall of epithelial cells becomes distended and wrinkled as secreted oil accumulates beneath its surface. Oil secretion begins at the bud stage and lasts till the end of anthesis, that is 22 days, on average. Pollination does not influence oil production.

In *O. kruegeri*, trichomatous elaiophores are situated on the central part of the callus. Unicellular trichomes project from the epidermis cells. Their outer walls are covered by a thin cuticle. In the dense cytoplasm of the trichomes, small plastids with few starch grains occur, whereas subsecretory parenchyma cells contain amyloplasts with large starch grains and raphides. The oil is already produced at the bud stage, about one week before flower opening, and lasts till the end of anthesis.

Key words: elaiophore, structure, oil secretion, *Oncidium cheirophorum*, *Ornithocephalus kruegeri,* Orchidaceae

INTRODUCTION

Plants attract flower visitors by a range of products such as nectar and pollen, more rarely perfumes, resins, waxes or floral oils (B u c h m a n n , 1987; E n d r e s s , 1994). Eight angiosperm families (Orchidaceae, Iridaceae, Krameriaceae, Malpighiaceae, Primulaceae, Scrophulariaceae, Solanaceae, Cucurbitaceae) are known to secrete oils as the reward for their pollinators. Three additional families: Caesalpiniaceae, Melastomataceae and Gesneriaceae were previously included in this list but it is not still clear if their oil is a reward (V o g e l , 1974; B u c h m a n n , 1987).

The floral oils are secreted by structures called elaiophores. V o g e l (1974) recognized two different types of elaiophores: epithelial and trichomal ones. In Orchidaceae, both of them occur. Epithelial elaiophores are composed of one layer of glandular epidermis and subgladular parenchyma. Usually, they occur on the labellar callus (S i n g e r and C o c u c c i , 1999; S i n g e r et al. 2006). The floral oil is secreted directly onto the epidermis surface, or accumulates under the cuticle (B u c h m a n n , 1974; S i n g e r et al. 2006). Trichomal elaiophores consist of numerous glandular hairs. Secretion from trichomal elaiophores is easily accessible to a wide spectrum of visitors, but oil production is always lower than in flowers with epithelial elaiophores (V o g e l , 1974; B u c h m a n n , 1987).

The majority of oil-producing species rely exclusively on oil-collecting bees for pollination (S i l v e r a , 2002). Bees that harvest and use floral oils are found primarily in the families Apidae and Melittidae (B u c h m a n n , 1987; M i c h e n e r , 2000). Floral oil is collected usually by female bees, which use it mixed with pollen as provision for larvae, as nest construction lining their brood cells in the nest to waterproof them or as an adult food resource (B u c h m a n n , 1987; E n d r e s s , 1994; R a s m u s s e n and O l e s e n , 2000). Contrary to the nectar, bees do not collect and transport the floral oil with their mouth parts but with their legs or abdomen and these body parts are covered with special branched hairs (V o g e l , 1974; B u c h m a n n , 1987).

The lipoid non-volatile material secreted by oil-flowers is usually colourless and consists predominantly of diacyl- and triacylglycerols, saturated fatty acids, paraffins, and esters. Other components related with elaiophore exudates are diglycerides, amino acids, glucose, carotenoids, phenolics, glycosides, nonvolatile isoprenoides, and saponins (V o g e l , 1974; B u c h m a n n , 1987; R e i s et al. 2000, 2006; S i l v e r a , 2002). The

quantity of oil can vary among members of the same genus. The difference may also relate to the quality of oil and, for example, oil from *Sigmatostalix picturatissima* is more similar to the oil of *Ornithocephalus* sp. than of other *Sigmatostalix* species (S i l v e r a , 2002).

The structure of elaiophores is relatively obscure, and only epithelial elaiophores in four species have been studied in any degree of detail (Singer and Cocucci, 1999; S t p i c z y ń s k a and D a v i e s , 2007; S t p i c z y ń s k a et al. 2007). Therefore, data on the structure of elaiophores in Orchidaceae is still insufficient. The aim of this study is to describe the elaiophore structure of morphologically different members of Orchidaceae: *Oncidium cheirophorum* Rchb.f. and *Ornithocephalus kruegeri* Rchb.f. syn. *Ornithocephalus ciliatus* Lindl.

MATERIALS AND METHODS

Fresh flowers of *Oncidium cheirophorum* Rchb. f. and *Ornithocephalus kruegeri* Rchb.f. were obtained from the Botanical Garden of the Maria Curie-Skłodowska University (UMCS) in Lublin.

The position of the elaiophores in complete flowers of *Oncidium cheirophorum* Rchb.f. and *Ornithocephalus kruegeri* Rchb.f. was determined using an Olympus SZX12 stereo-microscope. Hand-cut sections through the living elaiophore were tested for lipids and starch using saturated alcoholic Sudan III solution and Lugol solution, respectively. Stained sections were viewed with a Nikon H-III Rower light microscope (LM).

The elaiophores were fixed in 2.5% glutaraldehyde and 5% sucrose in phosphate buffer (pH 7.0; 0.1M) for 2h at 20°C, after that in phosphate buffer for 24h at 4°C, post-fixed in 1% osmium tetroxide at 4°C for 15 min. and washed in distilled water two times for 5 min. The fixed material was then dehydrated in ethanol, infiltrated and embedded in LR White resin, according to detailed producer protocol (London Resin Company Limited, UK).

Semi-thin sections (about 0.7 µm thick) were cut with a Reichert Ultracut S microtome and stained for general histology using 1% methylene blue and 1% azure II (1:1) for 5-7 min. Micrometry and photomicrography of the elaiophores were accomplished using a Jenaval or Nikon Eclipse 600 microscope.

For scanning electron microscopy (SEM), the elaiophores fixed as above, were dehydrated in acetone and critical-point dried using liquid CO_2. They were then sputter-coated with gold and examined by means of TESLA BS-300.

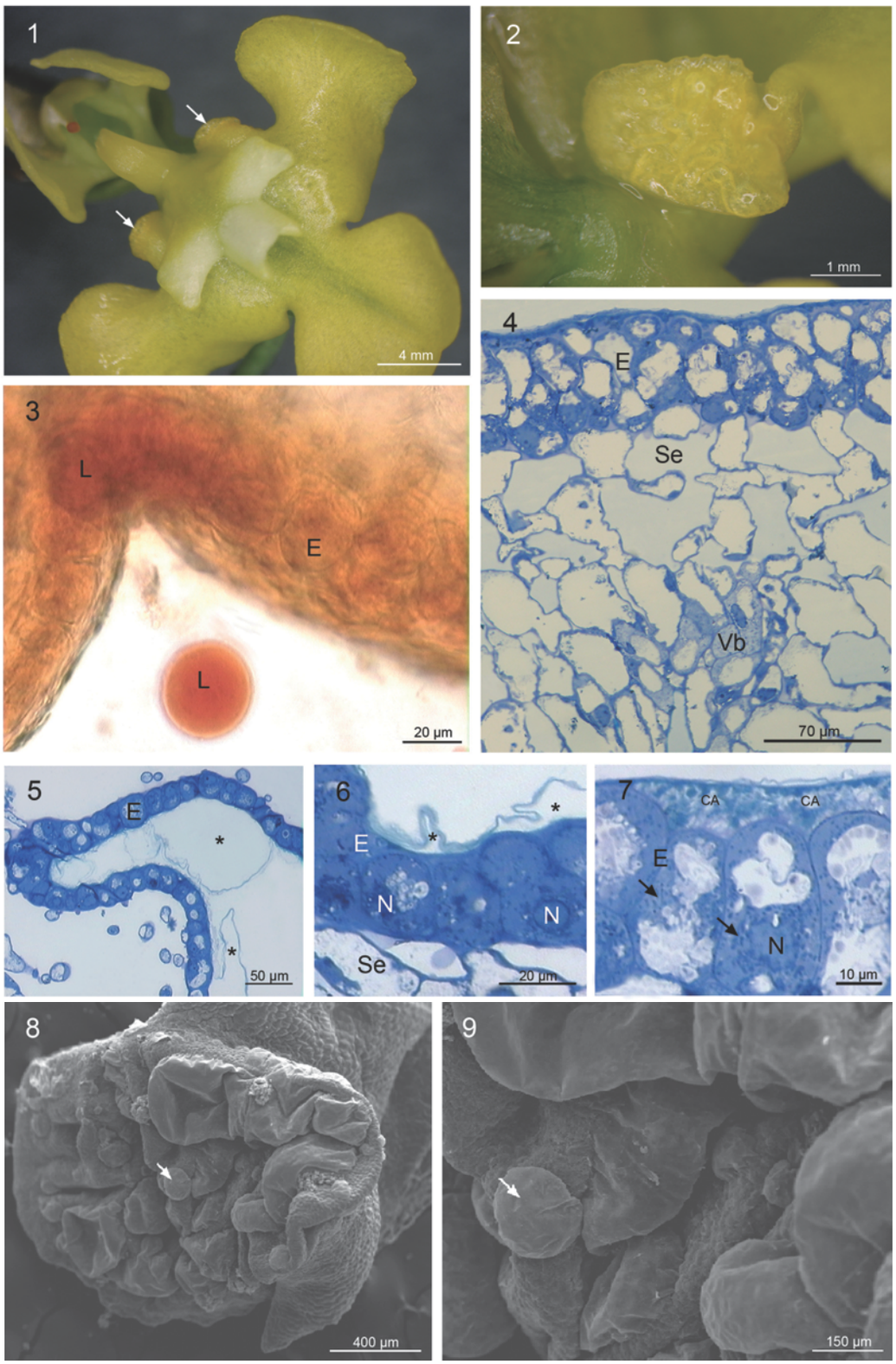
To estimate the weight of secreted oils, secretion accumulated at the surface of the flower was absorbed into filter paper strips, previously extracted with acetone and weighed. Measurements were conducted at bud stage, the eighth day of anthesis and daily, from 11th to 20th day of anthesis.

RESULTS

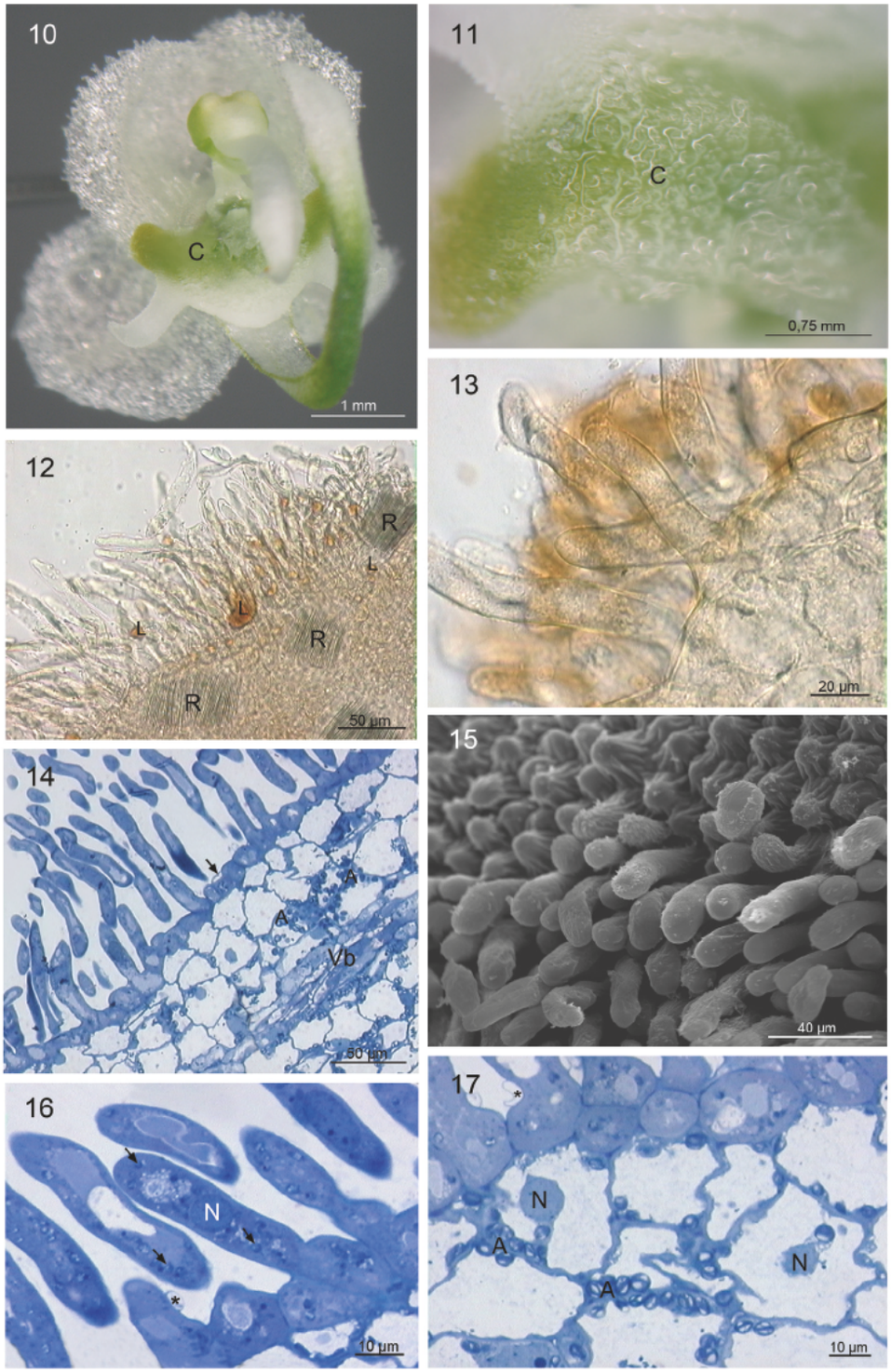
The flowers of *O. cheirophorum* are yellow, subtly fragrant and shining (Fig. 1). The size of each flower is about 1.5 x 1.2 cm. The average anthesis period is 22 days. The lip is three lobed and it has white callus in the central part from which the lobes branch out (Fig. 1). The elaiophores have a roundish structure with a crinkled surface, and their size is 2.0 x 1.5 mm (Fig. 2). They are situated symmetrically at the base of each lateral lobes, adjacent to the callus (Fig. 1).

The elaiophores are epithelial type and consist of single-layered cuboidal epithelium cells that measure 37 x 20 µm, on average (Figs 3-5). Under the epithelial layer, there are three layers of small subepithelial parenchyma cells and ground parenchyma with vascular bundles (Fig. 4). The epithelial cells contain dense cytoplasm and a large, centrally positioned nucleus, 9 µm in diameter, on average (Figs 6, 7). Treatment with IKI does not indicate the presence of starch in the plastids. In the thick outer cell wall, small numerous cavities occur (Fig. 7). Cavities do not occur in the thin, radial cell walls. The epithelial cell wall is covered by a thick cuticle of mean thickness of 1.4 µm (Figs 5, 6). The oil, both on the surface and within the cytoplasm of the epithelial cells stains intensely with Sudan III (Fig. 3). The cuticle covering the outer, tangential wall distends as secreted oil accumulates beneath its surface (Figs 5, 6 and 8, 9). Oil secretion begins at the bud stage, about 10 days before flower opening, and lasts till the end of anthesis. During the course of anthesis, one flower secretes 0.27 – 0.48 mg of oil. The highest secretory activity was noted on the eighth day of anthesis. The pollination does not influence oil production.

The flowers of *O. kruegeri* are very small, measuring 5.0 x 4.0 mm, on average (Fig. 10). The period of anthesis is about 15 days. Tepals are covered by multicellular trichomes with an unicellular, roundish head at the tip. The labellum is whitish-green and elongated. The callus is also whitish-green, and semi-rounded in shape with two prominent outgrowths (Figs 10, 11). At anthesis, the whole surface of callus is covered by oily secretion (Fig. 11). Oil is produced by unicellular trichomes situated on the central part of the callus (Figs 12-15). The trichomes are quite small during the first day of anthesis and they grow successively up to 78 µm in length. The trichomes project from the epidermis cells (Figs 14, 16, 17). The oil, both inside the cells and on the surface of the trichomes, stains intensely with Sudan III (Figs 12, 13). Conversely to the previous species, cavities do not occur in the outer cell wall. The cell wall is covered by the cuticle but, unlike that of *O. cheirophorum*, the cuticle is thinner and measures about 0.3 µm. Also in *O. kruegeri,* the cuticle detach from the cell wall as secreted oil accumulates between it and the secretory epithelium, but in a lesser degree that



Figures 1-9. *Oncidium cheirophorum*. Fig. 1. Flower of *O. cheirophorum* with elaiophore located at the base of each lateral lobe (arrows). Fig. 2. Elaiophore coated with secreted oil. Fig. 3. Section of elaiophore stained with Sudan III showing intracellular oil and lipid droplet on the surface. Fig. 4. Paradermal section of elaiophore showing epithelial layer, subepithelial parenchyma and ground parenchyma with vascular bundle. Figs. 5, 6. Epithelial cells with distended cuticle (asterisks). Fig. 7. Epithelial cells with central nuclei, leucoplasts (arrows) and thick outer cell wall with numerous cavities. Fig. 8. Wrinkled surface of elaiophore. Arrow indicates distended cuticle. Fig. 9. Details of elaiophore surface with distended cuticle (arrow).



Figures 10-17. *Ornithocephalus kruegeri*. Fig. 10. Flower of *O. kruegeri* with elaiophore on central part of callus. Fig. 11. Trichomatous elaiophore coated with oil. Fig. 12. Elaiophore tissue stained with Sudan III with oil accumulated on the surface of the cells. Subepidermal cells with raphides. Fig.13. Lipids stained with Sudan III inside secretory trichomes. Fig. 14. Unicellular trichomes with distended cuticle (arrow) and subepidermal parenchyma with amyloplasts. Fig. 15. Surface of secretory trichomes. Fig. 16. Trichomes with centrally positioned nucleus surrounded by plastids with starch grains (arrows) and distended cuticle (asterisk). Fig. 17. Elaiophore with distended cuticle (asterisk) and amyloplasts accumulated in subepidermal cells.

Abbreviations: A – amyloplast; C – callus; CA – cell wall cavity; E – epithelial cells ; L – lipid droplet; N – nucleus; R – raphide; Se – subepithelial layer; Vb – vascular bundle.

in the previous species. The detachment of the cuticle was predominantly observed at the base of trichomes (Figs 14, 16, 17). In the dense cytoplasm of the trichomes, small plastids with a few starch grains and lipid droplets occur. The cells have a large, centrally positioned nucleus, 10 μm in diameter, on average (Fig. 16). The cells of subepidermal parenchyma contain several amyloplasts with large starch grains (Fig. 17). Some of subepidermal cells accumulate raphides in their vacuoles (Fig. 12). In the deeply positioned ground parenchyma, vascular bundles occur (Fig. 14).

The secretory activity of trichomatous elaiophores begins before anthesis. Even though the callus is not fully developed, the oil is already produced at the bud stage, about one week before flower opening. The quantity of oil increases almost till the end of anthesis. One flower produces maximum 0.1 mg of oil.

DISCUSSION

Elaiophores of *Oncidium cheirophorum* and *Ornithocephalus kruegeri* are well-defined glands, similarly as in other oil-producing Oncidiinae (Stpiczyńska et al, 2007; Stpiczyńska and Davies, 2007). However, in certain other oil-producing orchids, such as *Gomesa recurva*, no obvious morphologically differentiated elaiophore is visible, even though oil is copiously produced (Stpiczyńska et al. 2007).

Elaiophores of *O. cheirophorum* and *O. kruegeri* are morphologically and anatomically diverse. Secretory glands in both investigated species are located on the labellum, but their detailed position evidently differs. In *O. cheirophorum*, elaiophores occur symmetrically on the lateral lobes of the lip, likewise in *Oncidium trulliferum, Trichocentrum cavendishianum* and *Oncidium paranaense,* whereas the callus is responsible for oil secretion in *O. krugeri,* similarly as in *Oncidium loefgrenii* (Singer and Cocucci, 1999; Stpiczyńska et al. 2007; Stpiczyńska and Davies, 2007). In *O. cheirophorum*, the elaiophore is of the epithelial type and consists of a single layer of cuboidal-like secretory cells and, additionally, several layers of subepithelial parenchyma which presumably supports the secretory activity of the epithelial cells. A similar anatomical organization occurs in *O. trulliferum, T. cavendishianum* and *O. loefgrenii* (Stpiczyńska *et al*, 2007; Stpiczyńska and Davies, 2007). The elaiophore of *O. kruegeri* is of the trichomal type, and to date, this study is the first report on the structure of the trichomal elaiophore in orchids. Interestingly, the secreting hairs in *O. kruegeri* are just one-celled, whereas in species such as *Nierembergia* (Solanaceae) (Cocucci, 1991) or *Colpias mollis* (Scrophulariaceae) there are a few cells (Steiner and Whitehead, 2002).

The structure of elaiophore cells in both investigated species is typical for secretory cells (Fahn, 2000). They have dense intensively staining cytoplasm and a large, centrally positioned nucleus. Similarly as in other elaiophores, the cells contain lipid droplets that are found scattered throughout the cytoplasm. Additionally, in the cytoplasm of *O. kruegeri* plastids with starch grains occurred. Amyloplasts are noted also in subepidermal parenchyma. The presence of starch is characteristic for many nectary and osmophore cells, generally at the presecretory stage, and it can exist as a source of energy for intensive metabolic processes or, indirectly, as nectar sugar components. In the elaiophores of previously studied species, starch was noted only in subsecretory parenchyma of *Ornithophora radicans* (Stpiczyńska and Davies, 2007).

Similarly as in the previously examined epithelial elaiophores of orchids (Stpiczyńska et al. 2007; Stpiczyńska and Davies, 2007) and malpighiaceous *Dinemandra ericoides* (Cocucci et al. 1996), LM observations show the presence of cavities in thick outer tangential cell walls in *O. cheirophorum*. However, the cavities are absent in *O. kruegeri*. It is thought that cavities may aid the transport of hydrophobic components across the hydrated cell wall, much in the same way that such compounds are transported during the formation of cuticle (Kunst and Samuels, 2003 and references therein). The structure of the cell wall in both species will need detailed studies in TEM.

The cuticle covering secretory tissues becomes distended as secreted oil accumulates beneath its surface. This feature is common for both examined species, regardless of elaiophore type, and it is probable that full discharge of oil is not completed until the cuticle is ruptured by a visiting insect. Distension of the cuticle is also known to occur in *T. cavendishianum* (Davies and Stpiczyńska, 2007; Stpiczyńska et al. 2007) and *D. ericoides* A. Juss (Malpighiaceae), which is pollinated by species of *Centris* (Cocucci et al. 1996).

In both examined orchids, secretion of lipids begins at the bud stage and the secretory activity lasts to the end of flower lifespan. The presence of lipids secreted in closed buds facilitates covering the surface of labellum and other tepals with lipids. Perhaps the shining appearance of the flowers provides additional advertisement for pollinators.

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Budowa elaioforów u *Oncidium cheirophorum* Rchb.f. i *Ornithocephalus kruegeri* Rchb.f. (Orchidaceae)

Streszczenie

Prawie cała powierzchnia okwiatu *Oncidium cheirophorum* i *Ornithocephalus kruegeri* jest pokryta błyszczącą, tłustą wydzieliną. Tłuszcze są produkowane i wydzielane przez elaiofory – specjalne gruczoły położone symetrycznie przy podstawie warżki lub na kallusie.

U *O. cheirophorum* występuje elaiofor epitelialny. Jest on zbudowany z jednej warstwy sześciennych komórek wydzielniczych i miękiszu podwydzielniczego. Gruba kutykula pokrywająca ściany zewnętrzne komórek epitelialnych odkleja się i marszczy pod wpływem gromadzącej się pod nią wydzieliny. Sekrecja tłuszczu rozpoczyna się w stadium pąka i trwa do końca antezy, to jest średnio 22 dni. Zapylenie nie wpływa na wydzielanie tłuszczu.

W przypadku *O. kruegeri* trichomowy elaiofor znajduje się w centralnej części kallusa. Jednokomórkowe włoski wydzielnicze wyrastają z komórek epidermy. Ich zewnętrzna ściana pokryta jest cienką kutykulą. W gęstej cytoplazmie trichomów znajdują się drobne plastydy z nielicznymi ziarnami skrobi, podczas gdy w komórkach podwydzielniczej parenchymy występują amyloplasty z dużymi ziarnami skrobi oraz rafidy. Sekrecja tłuszczu rozpoczyna się w stadium pąka, na tydzień przed otwarciem kwiatu i trwa do końca antezy.