ACTA BIOLOGICA CRACOVIENSIA Series Botanica 46: 193–200, 2004

TRANSFER CELL WALL ARCHITECTURE IN SECRETORY HAIRS OF *UTRICULARIA INTERMEDIA* TRAPS

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Received May 5, 2004; revision accepted July 12, 2004

This paper discusses transfer cell wall deposition and architecture in various trap hairs of the carnivorous plant *Utricularia intermedia*. Scanning electron microscopy showed that the middle cells of both internal hairs and pavement epithelium hairs have reticulate-type wall ingrowths. The wall ingrowths of the middle cell of both quadrifids and bifids are very well developed. However, in middle cells of pavement epithelium hairs the level of development of wall ingrowths is not uniform. The presence of ruptured cuticles and wall ingrowths in these hairs suggests that water is transported by the pavement epithelium hairs from the trap to the external environment.

Key words: *Utricularia*, transfer cell, wall ingrowths, hairs, carnivorous plants.

INTRODUCTION

Transfer cells occur in both generative and vegetative plant tissues (Gunning and Pate, 1974; Offler et al., 2003). For example, walls with ingrowths were recorded in elements of the embryo sac, including the central cell (Diboll and Larson, 1966; Wilms, 1981), antipodals (Bohdanowicz and Turała-Szybowska, 1985, 1987), synergids in *Spinacia* (Wilms, 1981) and even the egg cell of *Plumbago* (Cass, 1972). They are also found in endosperm cells (Davis et al., 1990; Nagl, 1992), suspensor cells (Schultz and Jensen, 1969; Bohdanowicz, 1987) and cotyledons (Talbot et al., 2001). Wall projections develop in vascular tissue, specifically in the xylem and phloem parenchyma (Pate and Gunning, 1969; Gunning et al., 1970; Gunning and Pate, 1974; Gunning and Steer, 1975; Talbot et al., 2002). Fineran and Calvin (2000) described wall projections in mistletoe sinkers. Transfer cells are frequent in different secretory tissues such as nectaries, glands and hydathodes (Fahn, 1979). It is commonly accepted that transfer cells are specialized in intensive short-distance transport between the symplast and the apoplast (Gunning and Pate, 1974). Transfer cells play a particularly important role in secretory glands and trichomes of carnivorous plant traps. These secretory structures not only attract animals and produce digestive enzymes but also must absorb the products of digestion (Juniper et al., 1989).

In contrast to the abundance of data on the occurrence of transfer cells, transfer wall architecture is poorly understood. The morphology of transfer walls was described in detail using scanning electron microscopy only in some species such as broad bean, corn and wheat (Talbot et al., 2001, 2002).

The aim of this work was to study the wall labyrinth architecture and ultrastructure in various trap hairs of the carnivorous plant *Utricularia intermedia* Hayne (Lentibulariaceae).

MATERIALS AND METHODS

Plants of *Utricularia intermedia* were obtained from four localities in SW Poland: Mikołeska, Strzybnica (Płachno 2003), Tworóg and the Jeleniak-Mikuliny Nature Reserve. For scanning electron microscopy

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PL ISSN 0001-5296

(SEM) traps were cut and fixed in 3% phosphate-buffered glutaraldehyde (GA) for 2 h at room temperature or in ethanol/acetic acid (3:1) and then dehydrated through an ethanol series. The material was critical-point dried using liquid CO_2. The dried tissues were coated with gold and viewed with a HITACHI S-4700 SEM in the Scanning Microscopy Laboratory of Biological and Geological Sciences of the Jagiellonian University. For transmission electron microscopy (TEM), traps were fixed for 2 h at room temperature in 3% phosphate-buffered GA, rinsed in buffer and postfixed in 1% osmium tetroxide in the same buffer for 2 h. The fixed material was rinsed in double-distilled water, dehydrated in ethanol/propylene oxide, and embedded in epoxy resin equivalent to Epon 812. The ultrathin sections were contrasted with uranyl acetate and lead citrate, and were viewed in a HITACHI H 500 electron microscope in the Department of Animal Histology and Embryology at the University of Silesia. Sections for light microscopy were cut at 0.5–2.0 μm with glass knives and stained with methylene blue in 0.5% sodium tetraborate. Autofluorescence observations of fresh sectioned traps were made. Histochemical localization of secretory products rich in polysaccharides with 1,2-glycol groups produced by certain glandular hairs was determined by the Schiff reaction (PAS).

RESULTS

Utricularia intermedia is a herbaceous carnivorous hydrophyte, which forms stolons with "leaves" and stolons with traps buried in the substrate for catching small organisms such as copepods and cladocerans. A single trap is a small ovoid bladder up to 4 mm in length, with an entrance and a stalk which attaches it to the plant (Fig. 1). The ventral part of the trap wall in the entrance forms the threshold. The inner surface of the trap is covered by quadrifid hairs (Fig. 2). Only the inner surface of the threshold is covered by bifid hairs (Fig. 3). Both quadrifids and bifids consist of a basal cell, a middle cell ('pedestal cell') and terminal cells, which have complex architecture. The proximal part of the terminal cell lies on the middle cell; the stalk is terminated by the arm (Fig. 3). The arms are free and project into trap lumen (Figs. 2 and 3). The nucleus, mitochondria and endoplasmic reticulum (ER) are located near the base of the arm (not shown). The middle and apical part of the arm lumen is vacuolated. In these parts, ER and numerous mitochondria are located in the cytoplasm between the vacuole and the cell wall. Perhaps the most interesting element is the middle cell, which is of both endodermoid and transfer character. This cell is essentially discoid in shape, though in transverse section it is round. The lateral walls of the middle cell are thick and impregnated by electron-translucent material (possibly cutin). The endodermoid walls resemble the epidermal cutinized wall (Fig. 6). The wall ingrowths arise mainly from the distal transverse wall and fill a large area of the cell lumen (Fig. 4). The wall ingrowths observed in SEM are branched, anastomosing finger-like structures (Fig. 5), which form a labyrinth. Observed in TEM, the wall projections consist of two different structural regions. They have a dense core surrounded by electron-translucent peripheral substance. The cytoplasm adjacent to the ingrowths contains mitochondria. The nucleus and small cisternae of rough ER occur near the transverse wall.

The trap threshold is built of large parenchyma cells and specialized epidermis. In transverse section, the threshold at the middle point shows three main parts: outer, inner and top. The outer part is covered by stalked glandular hairs, which produce mucilage-like substance rich in polysaccharides with 1,2-glycol groups (the terminal cells of these hairs exhibit a strong PAS-positive staining reaction). Bifid hairs lie in the inner part. Pavement epithelium is situated at the top of the threshold. Between the bifids and the pavement epithelium is a layer of large epidermal cells without hairs. This layer is absent in the lateral part of the threshold. The pavement epithelium is composed of sessile glandular hairs, which are closely packed. Each hair usually consists of a basal cell, a middle cell and two terminal cells. Occasionally there are two middle cells. The hairs of the anterior pavement epithelium have globose-shaped terminal cells and produce mucilage-like substance. In the posterior part of the pavement epithelium there are hairs with long terminal cells. Their cuticles are exfoliated and form velum, which seals up the trap door. The middle cell forms wall ingrowths on the transversal walls, both distal and proximal or only distal (Fig. 7). Wall ingrowths also occur at the terminal part of lateral wall in the corner between the lateral and transversal wall. The wall ingrowths are structures having reticulate morphology (Figs. 7, 8). However, one middle cell can have extensive wall ingrowths, whereas another may have a few wall ingrowths. In the former case, the wall ingrowths develop a laby-

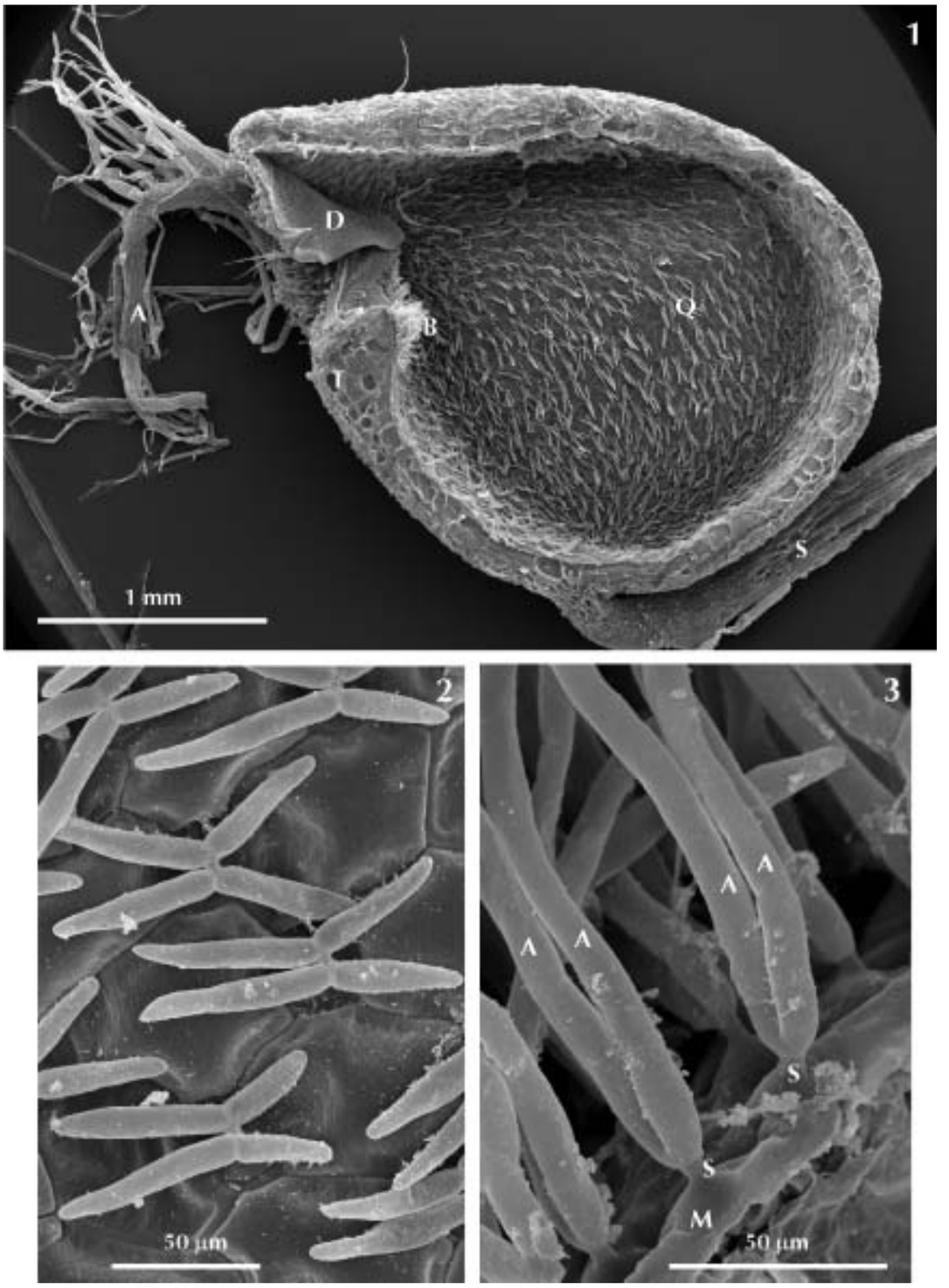

Fig. 1. Median section through trap of *Utricularia intermedia*. Q – quadrifid hairs; B – bifid hairs; T – threshold; asterisk – pavement epithelium; D – door; A – antennae; S – stalk. **Fig. 2.** Higher-magnification view of quadrifid hairs. **Fig. 3.** Higher-magnification view of bifid hairs. M – middle cell; S – stalk of terminal cell; A – arm of terminal cell. Figs. 1–3 SEM.

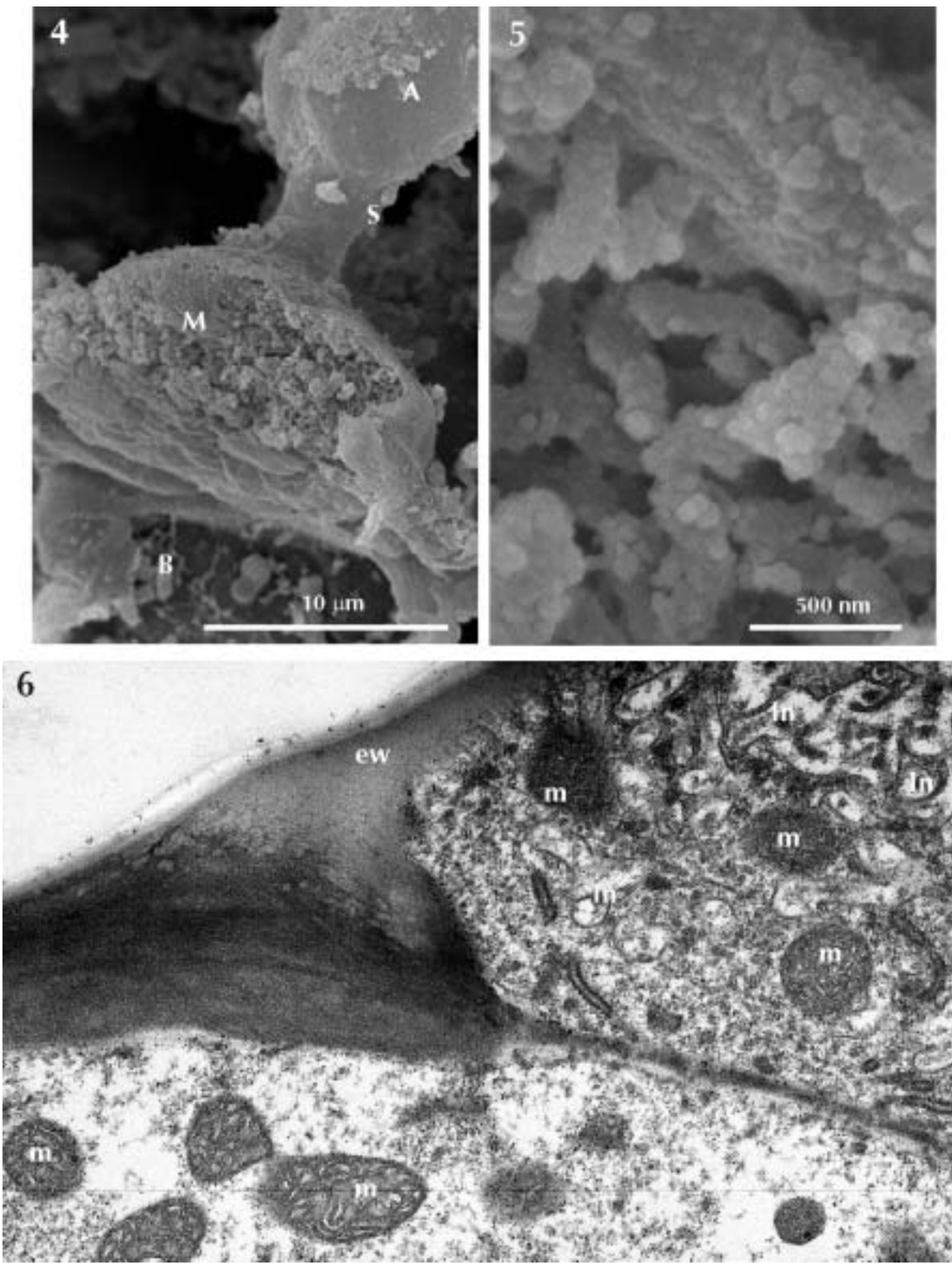

Fig. 4. Part of bifid hair. B – basal cell; M – middle cell; S – stalk of terminal cell; A – arm of terminal cell. Lumen of middle cell is filled with elaborate labyrinth of wall ingrowths. **Fig. 5.** Higher-magnification view of middle cell of bifid hair, showing open wall ingrowth architecture. **Fig. 6.** Longitudinal section of middle cell (top) and basal cell (bottom) of quadrifid hair. In – wall ingrowths; ew – impregnated lateral wall; m – mitochondrion. × 32,000. Figs. 4–5 SEM; Fig. 6 TEM.

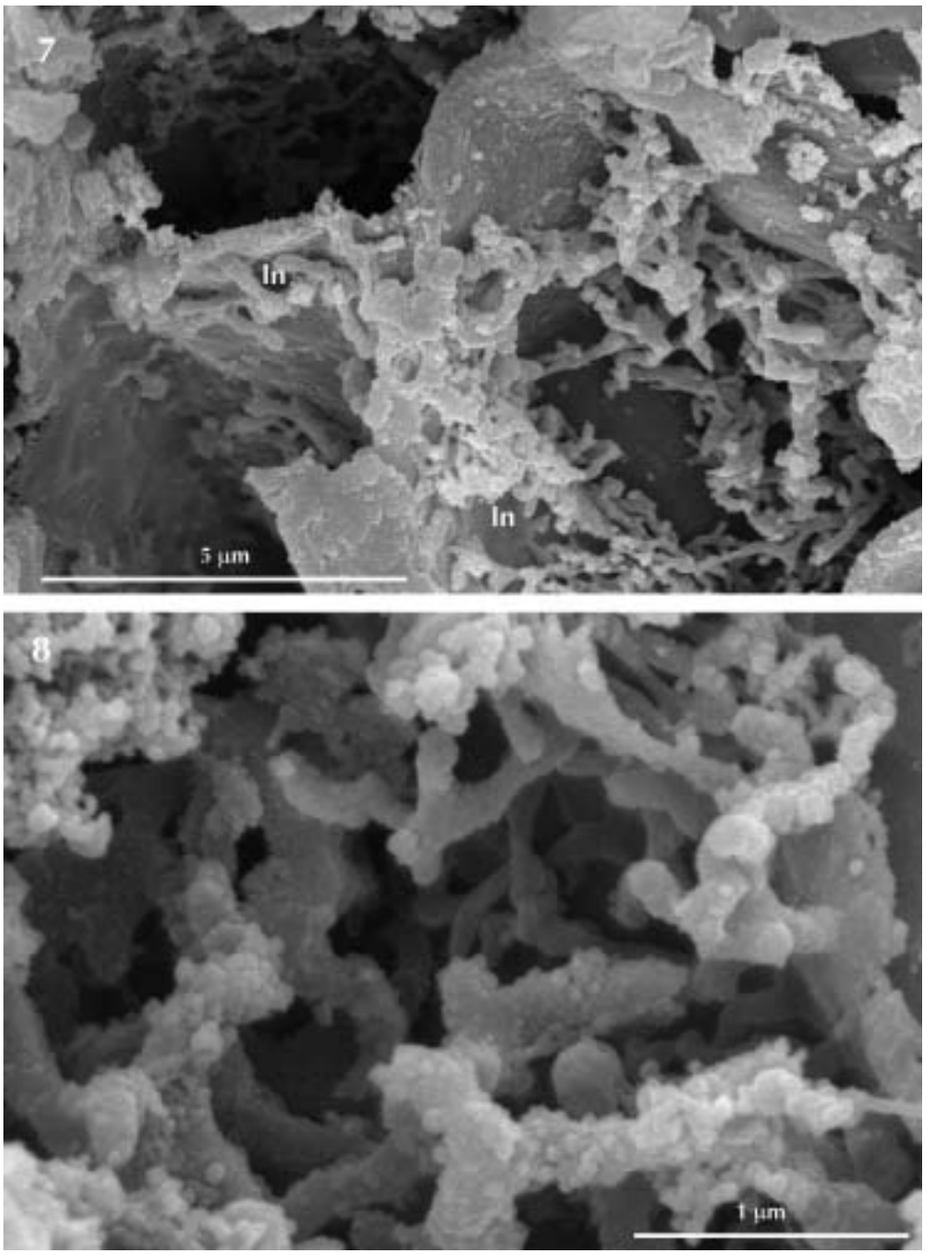

Fig. 7. Longitudinal section of a basal cell and middle cells of pavement epithelium hairs, showing wall ingrowths (In).
Fig. 8. Higher-magnification of wall ingrowths in middle cells of pavement epithelium hair.

rinth, which has a porous 'open' appearance and fills a large amount of the cell lumen. The wall ingrowths of the distal transversal wall can join with ingrowths of the proximal transversal wall (Fig. 7). In the latter case, the wall ingrowths form only a short network. In some hairs, wall ingrowths were not observed at all in the middle cells.

In some hairs, wall ingrowths occur also in the basal cells. In this case the wall ingrowths have morphology similar to those of the middle cell and occur specifically in the distal part of the cell.

DISCUSSION

Since Darwin's INSECTIVOROUS PLANTS (1875), species of *Utricularia* have drawn the attention of many botanists. However, there are only a few detailed studies of the organization and ultrastructure of secretory hairs of *Utricularia* traps. Fineran and Lee (1974a,b) were the first to show that the middle cells ('pedestal cells') of different glandular hairs of *Utricularia* traps were transfer cells. In the same year, however, Beltz (1974) mentioned wall ingrowths in the middle cells of outer glands in the trap of *U. macrorhiza* Le Conte. Later, Ghirardelli Gambardella and Honsell (1975) described the middle cell of inner glandular hairs of *U. vulgaris* L. as transfer cells. Fineran and Lee (1974a,b; 1975) and Fineran (1985) used transmission electron microscopy and high-voltage transmission electron microscopy to produce the first description of well-developed wall ingrowths in pedestal cells of quadrifids and bifids in *U. monanthos* J. D. Hook. In this species, the wall ingrowths arise mainly from the inner transverse wall and distal walls, and have 'branched ramifying tube-like morphology' (Fineran 1985 p. 309). Numerous mitochondria occur in the cytoplasm between ingrowths. In *U. intermedia* we also observed intensive wall ingrowths in pedestal cells of quadrifids and bifids. The morphology of the wall ingrowths was similar to that in *U. monanthos*, though the mitochondria situated close to ingrowths were less numerous than in *U. monanthos*. Talbot et al. (2002) examined the morphology of transfer cells from eleven species of monocotyledons and dicotyledons. Based on that study, two main categories of architecture of wall ingrowths were recognized: reticulate and flange. In *U. intermedia*, the architecture of wall ingrowths in the middle cells of internal glandular hairs (quadrifids, bifids) could be classified as reticulate. The morphology of wall ingrowths in the middle cells of pavement epithelium glandular hairs fits the same category. Fineran and Lee (1974b) reported transfer walls in the middle cells of the pavement epithelium of the terrestrial, primitive *U. monanthos*. However, Beltz (1975) did not observe them in the pavement epithelial cells of aquatic *U. macrorhiza*, which belongs to a section of *Utricularia*: 'This appears to be at least a species difference and may represent a difference characteristic between aquatic forms such as *U. macrorhiza* and terrestrial forms such as *U. monanthos*.' (1975, p. 125) If this is true, it may be expected that wall ingrowths in the middle cells of pavement hairs should not be found in other aquatic species from the same section. However, in the pavement epithelium of aquatic species like *U. vulgaris*, *U. australis* R. Br. (Broussaud and Vintjoux, 1982) and *U. intermedia* (this work), transfer cells have been reported, though the complexity and number of wall ingrowths have varied. This is due not only to the specialization of different zones of the pavement epithelium, as pointed out by Broussaud and Vintejoux (1982). This modification of transfer cell wall architecture seems also to reflect the physiological state of the cells. Fineran and Lee (1975) suggested that the appearance of the labyrinth wall in the middle cell of quadrifids and bifids of *U. monanthos* depends on the activity of the trap. It should be noted that wall ingrowths in plant cells can be developed in a relatively short time (Farley et al., 2000; Talbot et al., 2002) and can be rapidly removed (Briggs, 1995). The complexity of wall ingrowths is directly correlated with the intensity of transport by the transfer cell (Gunning and Pate, 1969). This means that transfer cell wall architecture is mobile and depends on both the physiological and developmental state of the cell.

It is believed that the internal hairs in the trap of *Utricularia* have three main functions: producing enzymes, absorbing digestive products, and removing water from the trap lumen during the resetting of the trap (Fineran 1985; Juniper at al., 1989). Enzymes like protease, acid phosphatase and esterase were found long ago in the digestive hairs of *Utricularia* (Vintéjoux, 1974; Heslop-Harrison, 1975). Recently, Sirová et al. (2003) used enzyme-labeled fluorescence to detect phosphatase activity in quadrifids in *U. australis* and *U. ochroleuca* s.l. The same authors also showed in situ phosphatase activity in trap fluid. It seems that the terminal cells of quadrifids play a major role in producing enzymes for digestion of prey. This activity is probably connected with rough endoplasmic reticulum. In contrast to the terminal cell, the middle cell in a mature hair has a small amount of endoplasmic reticulum. However, this cell has both transfer and endodermal features, and plays a key role in the absorption of water from the trap lumen (Fineran, 1985). Wall ingrowths increase the surface area of the plasma membrane, in turn increasing the transport of water and solutes. Moreover, the endodermoid lateral wall of this cell gives the cell total control over transport. Fineran and Gilbertson (1980) demonstrated this using lanthanum and uranyl salts, showing the lateral wall to be completely impregnated (a true apoplastic barrier).

Richards (2001), who studied trap content in *U. purpurea*, suggested that this species benefits more from mutualism than from predator-prey interaction. In this situation the internal hairs absorb nutrients both from digested animals and from the "community of microorganisms" in the traps. These results are very interesting because mutualism interactions are known from other carnivorous plants: pitcher plants (Juniper et al., 1989), *Drosera* and *Byblis* (Lowrie, 1998). However, *U. purpurea* belongs to the anomalous section *Vesiculina*. For example, the members of this section have uniquely constructed trigger hairs (Taylor, 1989). Moreover *U. purpurea* is a free-floating macrophyte, in contrast to *U. intermedia* which is affixed-aquatic with traps often buried in the substrate. Nevertheless, it will be very interesting to study "carnivorous" interactions in species from different sections and to make comparisons with the results in Richards' (2001) paper.

What is the function of transfer cells in the hairs of the pavement epithelium? It has been pointed out that these hairs produce mucilage (Broussaud and Vintéjoux, 1982; Fineran, 1985) or velum (Lloyd, 1942; Broussaud and Vintéjoux, 1982). Both types of hairs were detected in *U. intermedia*. Sydenham and Findlay (1975) showed that immersing a trap in paraffin oil made it is possible to distinguish the fluid from the trap lumen emerging from the mouth region while it is resetting. Fineran and Lee (1975) and later Sasago and Sibaoka (1985) suggested that water absorbed by bifids could be excreted by the hairs of the pavement epithelium. The ruptured cuticle and wall ingrowths may be involved in this process.

ACKNOWLEDGMENTS

We thank Professor Jerzy Klag for giving us the opportunity to use the transmission electron microscope, the staff in the Department of Animal Histology and Embryology at the University of Silesia for their help, Professor Elżbieta Pyza for giving us the opportunity to use the scanning electron microscope, and Jadwiga Faber for technical assistance in the use of the scanning electron microscope. The research was funded by the Jagiellonian University, grant no. DBN-414/CRBW/K-VI-15/2003.

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