ANATOMY AND ULTRASTRUCTURE OF FLORAL NECTARY OF *INULA HELENIUM* L. (ASTERACEAE)

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ABSTRACT

Floral nectaries of *Inula helenium* L. only occurred in disc florets and were situated above the inferior ovary. The shape of the investigated glands (five-armed star with rounded tips and deep incisions – observed from above) clearly differed from the shape of the nectaries of other Asteraceae, also the height of nectary was much lower (129 µm). The glandular tissue of the nectaries of elecampane was composed of a single-layered epidermis and 5--9 layers of secretory cells. Nectar was released through modified stomata, mainly arranged in the top part of the gland. The secretory cells were characterised by granular cytoplasm and the presence of a large, often lobate, cell nucleus. In the cytosol, numerous amoeboid plastids, mitochondria, Golgi bodies and ribosomes were present. In small vacuoles, myelin-like structures, fibrous material and vesicles with the content of substances which can be secretion, were observed. The plastid stroma showed different electron density and the presence of internal tubules and plastoglobules. Vesicular extensions forming bright zones were visible between the membranes of the nuclear envelope. Adjacent to the plasmalemma, as well as between the plasmalemma and the cell wall, secretory vesicles occurred, indicating the granulocrine mechanism of nectar secretion.

KEY WORDS: Asteraceae, *Inula helenium*, nectary, micromorphology, stomata, anatomy, ultrastructure.

INTRODUCTION

Inula helenium L. (Asteraceae) is a high perennial plant (1.5-2.0 m) originating from Central Asia. This plant is used as a medicinal, spice, melliferous and ornamental plant. It is often found as a cultivation relic nearby old palace and monastery buildings. Yellow flower heads of elecampane develop in June-August, growing individually or in corymbs.

The topography, shape and anatomy of floral nectaries can be significantly different, even within one family (Frei 1955; Petanidou et al. 2000). Therefore, the structural features of nectar-secreting glands play a large role in determining plant affinity, and they can have a diagnostic value in plant taxonomy. The knowledge of the structures of nectaries is also important for apiculture on the account of correlations of the structure and abundance of nectar secretion.

Information on the structure of nectaries in representatives of the Asteraceae family is scarce. To date, the anatomy of these glands has been investigated in *Helianthus annuus* (Sammataro et al. 1985) and in several *Centaurea* species (Gulyás and Pesti 1966). Smets (1986) presented the location and shape of the nectaries in *Lindheimera texana* and Wróblewska (1997) described the morphology of the nectaries of *Silphium perfoliatum*. Literature data show that the nectaries of the Asteraceae are situated in disc florets at the base of the style (Kulijev 1959; Sammataro et al. 1985; Wróblewska 1997). According to Fahn (1952), nectar-secreting glands in this family can be classified as stylar nectaries. Smets (1986) includes them among nectaria persistentia, whereas Schmid (1988) in reproductive nectaries. Jabłoński (1998) states that the nectaries in the Asteraceae family are completely hidden, but situated not very deep in hemitropous flowers, thus, they are accessible for insects with mouthparts of average length and with long mouthparts. According to Fahn's (1953) reports, in phylogenetic development of plants, the nectaries change their position from the outer to inner parts of the flower and from the location at the base of the ovary to the base of the style. According to this, the nectaries of the Asteraceae can be included in among which have reached a high level in evolutionary development.

In literature no information was found on the structure of the floral nectaries of *Inula helenium*. The aim of this study was to gain knowledge on the morphology, anatomy and ultrastructure of the nectaries in this species.

MATERIAL AND METODS

The *Inula helenium* L. plants came from a collection of the Department of Vegetable Crops and Medicinal Plants of the Agricultural University of Lublin.

Flowers in full flowering phase were used for the study. At first the location of the nectaries was determined. In the disc florets, measurements were made of the length of the corolla (n = 20) and of the diameter of the corolla tube (n = 20) at the nectary level. The diameter and the height of the nectary gland (n = 10), the stomata width (n = 10), the height of the epidermal cells proper (n = 30) and the major diameter (dimension along the apical-basal axis) of the ellipsoid secretory cells (n = 30) were measured. Also the number of cell layers making up the nectariferous tissue was determined. The measured nectaries came from 10 florets produced by different inflorescences.

Preliminary observations of the nectaries were made by stereoscopic microscopy and by light microscopy. The micromorphology of the nectaries was examined by scanning electron microscopy. Features of ultrastructure of the secretory cells were analysed.

Scanning electron microscopy (SEM)

The surface of the nectaries, with special attention paid to the location of stomata, was examined by scanning electron microscopy. The disc florets with nectaries were fixed in 4% glutaraldehyde in 0.1 M phosphate buffer (pH 7.0) for 4 hours at room temperature. In the next stage, the material was dehydrated in ethanol series and acetone and dried at critical point with liquid CO_2 by using the Bal-Tec CPD 030, and coated with gold and platinum by using the Polaron SC 7640 sputter coater. Observations and photos were made in the BS 301 scanning electron microscope with the Tescan attachment for digital processing of microscope imaging.

Light microscopy

The anatomical analysis of the nectaries was based on semi-thin sections, 0.5 µm thick made from the longitudinal segments of disc floret. They were stained with 1% methylene blue with 1% azur II in a 1% aqueous solution of sodium tetraborate. The material was fixed and embedded in synthetic resin with the standard method used in transmission electron microscopy. Observations and photos were made by using the Jenaval Kontrast microscope, as well as the Eclipse 400, Nikon.

Transmission electron microscopy (TEM)

For ultrastructural examination, the nectaries were fixed in 2% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M cacodylate buffer with pH 7.4 at room temperature for 2 hours, and then for 12 hours at temp. 4°C. After washing the specimens for 24 hours in 0.1 M cacodylate buffer at temp. 4°C and postfixing them in 1% OsO_4, they were transferred to distilled water and stained in a 0.5% aqueous solution of uranyl acetate. Next, the material was dehydrated in alcohol series (ethanol) and propylene oxide. The specimens were embedded in Spurr Low Viscosity resin for 12 hours at temp. 70°C. The material was sectioned by use of the Reichert Ultracut S ultramicrotome. The ultrathin sections (60 nm thick) were treated with an 8% solution of uranyl acetate in 0.5% acetic acid and lead citrate. Observations of the secretory cells of the nectaries were made and their documentation was prepared by use of the BS-500 Tesla transmission electron microscope.

RESULTS

The pseudanthia of elecampane are composed of ray florets and disc florets (Fig. 1). The nectaries occurr only in the disc florets (Figs 2-3). The glands were situated at the level of the base of the corolla tube, at the top of the inferior ovary, surrounding the base of the style (Fig. 3). The nectaries, observed from above, had the shape of a five-armed star with rounded tips and deep incisions (Figs 4-5). Data collected in Table 1 show that the diameter of the nectary was 3.8 times larger than its height. Comparing the length of the corolla and the height of the nectaries, it can be seen that the nectaries accounted for 1.2% of the corolla length. The diameter of the corolla tube at the nectary level and the diameter of the gland show that the nectaries occupied 68.4% of the diameter space delimited by the corolla tube.

The examined nectaries were composed of a single-layered epidermis and 5-9 layers of glandular cells (Figs 7-8). The size and the shape of the secretory cells were clearly different from the cells of the tissues situated below the nectary. The glandular cells were smaller, had more dense cytoplasm and thus were more heavily stained. The shape of the secretory cells in the longitudinal section was spherical or oval. Twin cells, formed by the recent division, were often observed. The glandular tissue was characterised by a compact arrangement of cells. Nevertheless, observations of the nectariferous tissues in TEM showed the presence of small intercellular spaces (Fig. 10). Comparing the dimensions of the epidermal cells and of the secretory cells provided evidence that the size of both types of cells was similar (Table 1; Fig. 8).

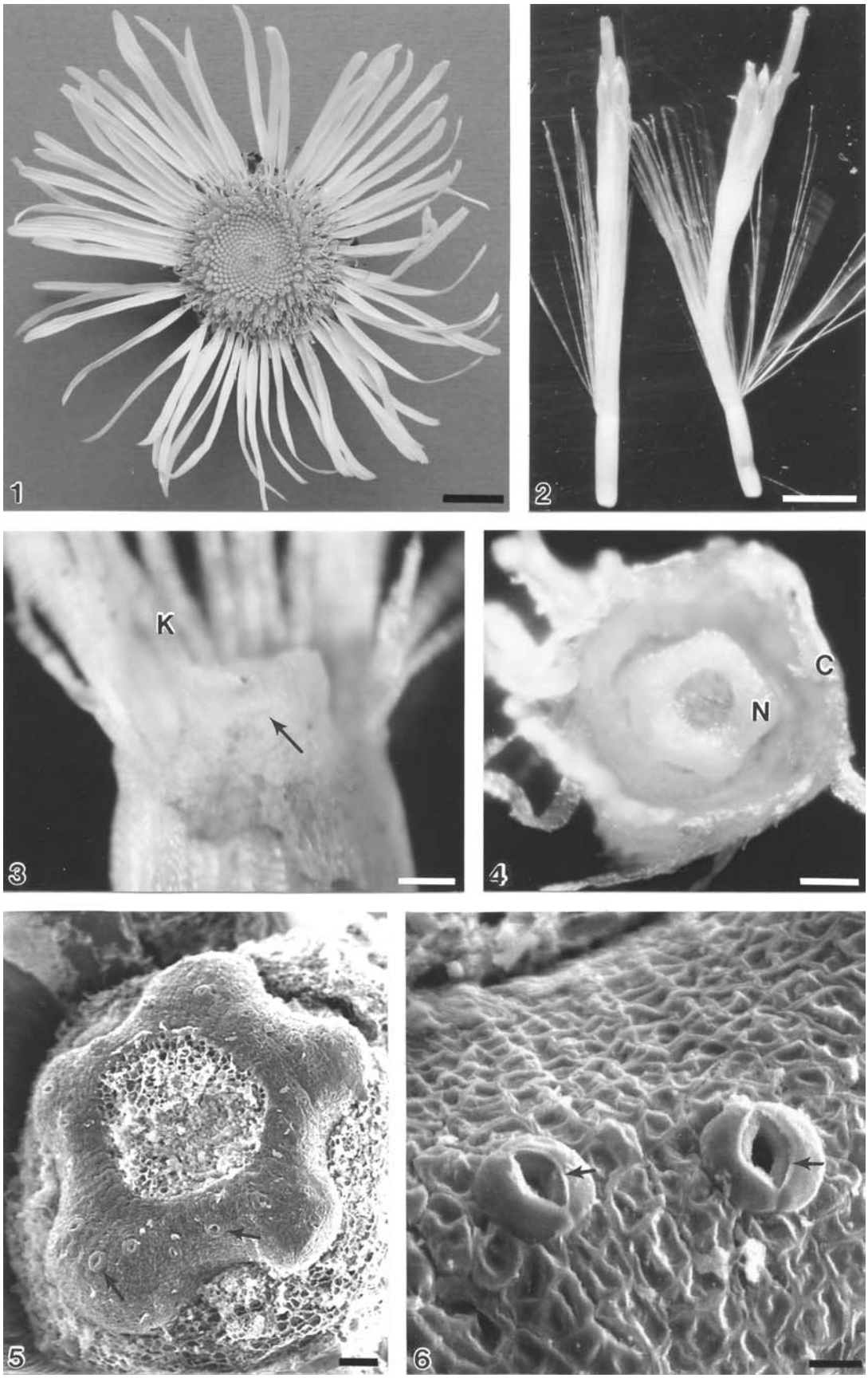
No vascular bundles were observed in the secretory tissue area, only in the subglandular tissue the phloem and xylem strands were present, branching from the vascular bundles of the style and supplying the nectary (Fig. 7).

Nectar was secreted through modified stomata which were located mainly in the top part of the gland. The stomatal cells were of a kidney-like shape and they were elevated above the surface of epidermis. They were distinguished by larger sizes compared to the other cells of the epidermis. On the side of the pore, small cuticular ledges were visible (Figs 5-6, 8). The walls of the epidermal cells were relatively thin. The stomatal apertures were quite large (Fig. 6), especially when observed in the longitudinal sections (Fig. 8).

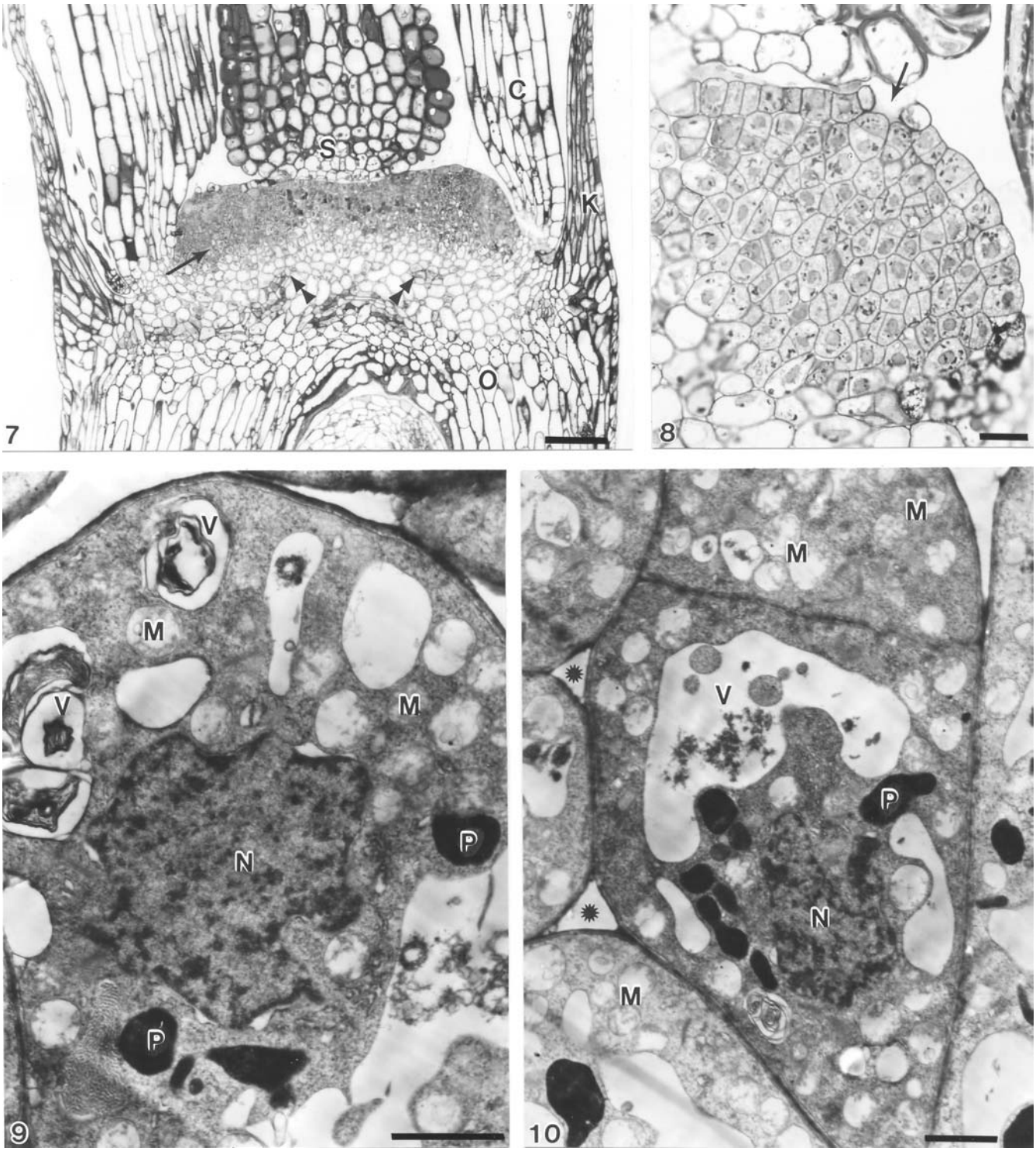
The secretory cells of the nectaries, analysed by means of transmission electron microscopy, were characterized by

TABLE 1. Some features of corolla and nectary structure in *Inula helenium* L. disc florets. All measurements are given as the mean value, SD and range.

Studied features	Results of measurements		
	Mean	SD	Range
Length of floret corolla (mm)	10.49	±0.4	10.17-11.22
Diameter of floret corolla (µm)	736.96	±67.06	652.17-834.78
Height of the nectary (µm)	128.50	±11.97	113.08-146.49
Outer diameter of the nectary (µm)	492.43	±16.31	475.47-521.73
Number of the gland cell layers	7.7	±1.16	5.0-9.0
Height of the epidermal cells (µm)	13.12	±1.57	11.43-16.33
Major diameter of the gland cells (µm)	13.38	±2.94	8.16-19.6
Width of stomata (µm)	49.0	±4.21	45.0-53.0

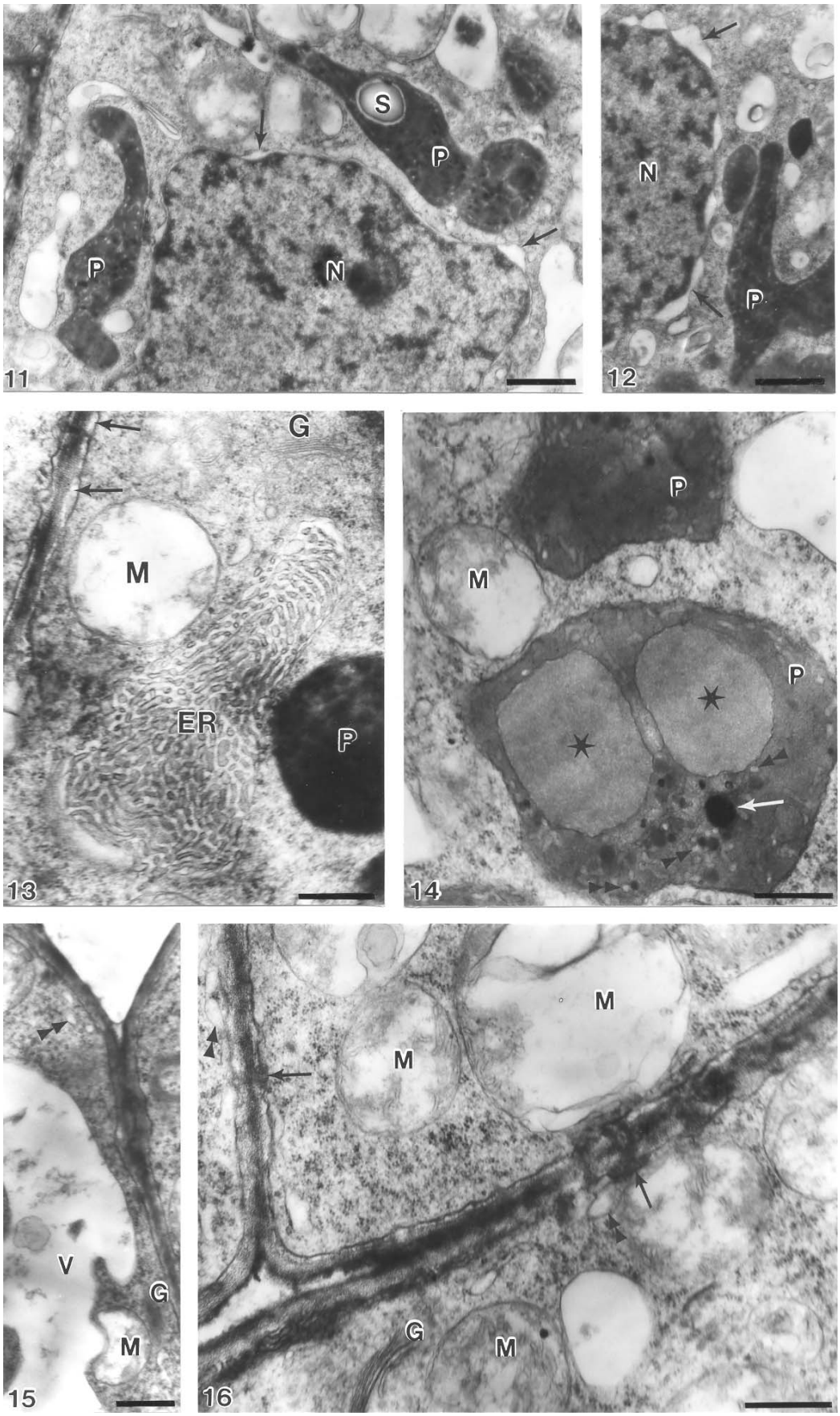


Figs 1-6. Fig. 1. Single inflorescence (anthodium) of *Inula helenium*. Bar = 1 cm. Fig. 2. Disc florets of elecampane. Bar = 2 µm. Fig. 3. Portion of the longitudinal section of disc floret with visible nectary (arrow) and elements of calyx (K). Bar = 200 µm. Fig. 4. Nectary (N) observed from above after removing the upper part of the corolla (C). Bar = 200 µm. Fig. 5. Nectary visible in SEM. Arrows show stomata. Bar = 50 µm. Fig. 6. Fragment of the surface of nectariferous tissue with stomata. Small cuticular ledges are visible on the side of the open pore (arrows). Bar = 20 µm.



Figs 7-10. Fig. 7. Portion of the longitudinal section of disc floret with visible nectary (arrow). S – style; C – corolla; K – calyx; O – ovary. Double arrowheads show endings of the vascular bundles at the base of the gland. Bar = 100 µm. Fig. 8. Fragment of the nectary with an open stoma (arrow) as seen on. Bar = 20 µm. Figs. 9-10. Cells of the lower layer of the nectary with visible lobate nuclei (N), vacuoles (V) containing deposits of different substances, amoeboid plastids (P) and numerous mitochondria (M). Between the cells intercellular spaces are present (asterisks). Bar = 2 µm.

thin walls, granular cytoplasm and the presence of a large, lobate cell nucleus. Local vesicular extensions forming bright zones were visible between the membranes of the nuclear envelope (Figs 11-12). In small vacuoles, myelin-like structures, fibrous material and vesicles with the content of substances, which could be a secretion, were observed (Figs 9-10). In the mesoplasm of the glandular cells, numerous pleomorphic plastids occured. The plastid stroma was marked by varied, quite significant osmophilicity and by the presence of plastoglobules, intraplastid, irregularly arranged tubules, as well as bright membranous zones (Figs 11-14). Sometimes, starch was also visible within the plastids (Fig. 11). In the secretory cells, numerous Golgi bodies and mitochondria occurred (Figs 9-10, 15-16). The network of endoplasmic reticulum structures was very extensive. The endoplasmic reticulum profiles were concentrated near the nuclear envelope, plastids and mitochondria. They were also present in close proximity to the plasma-



Figs 11-16. Figs. 11-12. Fragments of glandular cells with bright, expanded perinuclear zones between membranes of nucleus envelope (arrows), pleomorphic plastids (P) with stroma of different electron density and starch grains (S). Bar = 1 µm. Fig. 13. Profiles of endoplasmic reticulum (ER) make characteristic clusters near mitochondrium (M), plastid (P) and Golgi bodies (G). Secretory vesicles are visible between plasmalemma and the cell wall (arrows). Bar = 0.5 µm. Fig. 14. Plastid with the big, lighter zones (asterisks), internal tubules (double arrowheads) and osmiophilic globules (arrow). Bar = 0.5 µm. Figs. 15-16. Walls of adjacent secretory cells penetrated with plasmodesmata (arrows). Secretory vesicles are visible (double arrowheads) close to the plasmalemma. In protoplasts mitochondria (M), Golgi bodies (G) and numerous ribosomes are present. Bar = 0.5 µm.

lemma (Figs 11-16). The endoplasmic reticulum tubules locally formed characteristic clusters near the mitochondria and plastids (Fig. 13). Free ribosomes and ribosomes bound to the endoplasmic reticulum profiles were also located in the cytoplasm of the glandular cells. The walls of the observed cells were electron dense and contained plasmodesmata (Figs 15-16). The occurrence of plasmodesmata indicate the symplastic transport of pre-nectar. The plasmalemma was corrugated and its separation from the cell wall was often observed. In the cytoplasm, adjacent to the plasmalemma and between the plasmalemma and the cell wall, numerous secretory vesicles of different size were visible (Figs 13, 15-16). Their presence can be related to the granulocrine mechanism of nectar secretion.

DISCUSSION

The nectaries of *Inula helenium* were found to be located only in the disc florets. They were situated above the ovary at the base of the style. A similar topography of the nectaries in other representatives of Asteraceae was described by Kulijev (1959), Gulyás and Pesti (1966), Sammataro at al. (1985) and Wróblewska (1997).

Literature contains few data on the shape and dimensions of the nectaries in representatives of Asteraceae family. Gulyás and Pesti (1966) attribute to the nectaries of cornflower the shape of a tube or funnel of height 0.6-1.8 mm. In sunflower, discoid nectaries were observed, which in cultivated genotypes were 200-360 µm high and had the inner diameter of 470-800 µm, whereas in wild growing species these dimensions were of 273 µm and 133 µm, respectively (Sammataro et al. 1985). When comparing the literature data with the results related to the nectaries of elecampane, it can be stated that the shape of the glands investigated in the study clearly differed from the shape of the nectaries described in literature for this family. The diameter of the nectary of elecampane (490 µm) was close to that given for nectaries in cultivated forms of sunflower, but the nectary reached a much lower height (129 µm) than the nectaries of other Asteraceae.

The structure of nectaries of elecampane corresponds to the general structure of floral nectaries (a single-layered epidermis, several layers of glandular cells) in other plants (Figueiredo and Pais 1992; Weryszko-Chmielewska 2000; Weryszko-Chmielewska et al. 2003). The differences relate to the size and shape of the cells, the compactness of their arrangement, the number of layers of secretory cells, their ultrastructure and the venation of the nectary gland. The thickness of the secretory tissue of a small nectary of elecampane was 8 layers on the average. The mean length of its secretory and epidermal cells was 13 µm. Information on the size of the secretory cells is only given by Gulyás and Pesti (1966) for the glands of cornflower (10-20 µm). In the literature no information has been found on the size of cells of the nectaries in other Asteraceae.

Descriptions in literature show that nectar can be secreted outside through the walls of the secretory cells (Rachmilevitz and Fahn 1973; Pais and Figueiredo 1994), of trichomes (Sawidis et al. 1989; Sawidis 1998), or it can be released through the modified stomata (Nepi et al. 1996; Weryszko-Chmielewska et al. 2003; Weryszko-Chmielewska et al. 2004; Davis et al. 2005). In the nectaries of elecampane, nectar was secreted through the stomata. Gulyás and Pesti (1966) and Sammataro et al. (1985) also proved stomatal nectar secretion in cornflower and sunflower. The width of the stomata in elecampane reached 45-53 µm. The size of the stomata in *Centaurea* was 20-40 µm (Gulyás and Pesti 1966), whereas the width of such stomata in sunflower was 24-45 µm (Sammataro et al. 1985). The cited data show that the stomata of elecampane were the largest.

Frei (1955) states that the nectaries of the Asteraceae may have no vascular bundles, they can have only phloem or both: xylem and phloem strands. Studies of Sammataro et al. (1985) revealed in the nectariferous tissue the presence of phloem elements which reached close to the stomata. In the species investigated here, vascular bundles ran up only to the style and had their endings in the subglandular tissue. Knowing the correlation between the vasculature of the nectariferous tissue and the composition and amount of nectar produced (Frey-Wyssling and Agthe 1950), one can assume that the nectaries of elecampane produce nectar with a low concentration of sugars.

The protoplasts of the glandular cells, observed in transmission electron microscope, showed clear features of secretory structures. In literature there is no information on ultrastructure of nectary cells in the Asteraceae. Certain features of glandular cells of the investigated species are similar to those described for nectaries of plants belonging to other botanical families (Fahn 2000). In secretory cells of the nectaries of elecampane, amoeboid plastids occurred with high frequency. In their stroma the tubular structures, osmiophilic plastoglobules and brighter zones were visible. Stpiczyńska (1997, 1999) observed the presence of plastoglobules and the internal reticulum in cells of the osmophores of *Platanthera chlorantha*. Intraplastid tubular elements in the plastid stroma were also noticed in glandular trichomes of *Achillea millefolium* (Cheniclet and Carde 1985; Figuiredo and Pais 1994) and *Teucrium scordonia* (Sevinate-Pinto and Antunes 1991). In certain plastids of the nectaries of elecampane, starch grains were visible. As stated by Nepi et al. (1996), this polysaccharide can be used as a source of energy for metabolic processes or as a source of sugars in the synthesis of nectar.

In the cytoplasm of secretory cells of the nectaries of elecampane, an extensive network of endoplasmic reticulum profiles was observed. According to Fahn's (1979) reports, the rough endoplasmic reticulum structures are involved in the transport of pre-nectar in the nectariferous tissue and in the accumulation of nectar in secretory cells. In our case, the profiles of the endoplasmic reticulum sometimes formed characteristic clusters adjacent to the mitochondria and plastids. Similarly looking reticulum profiles were observed in the nectariferous trichomes in *Hibiscus rosa-sinensis* (Sawidis et al. 1989). Numerous Golgi bodies were observed in cytosol of the nectariferous cells of elecampane. According to Fahn's (1979) reports, these organelles can participate in nectar storage. The walls of the nectariferous cells of elecampane were penetrated by plasmodesmata. Their presence may indicate the symplastic transport of nectar. In turn, the occurrence of intercellular spaces in the secretory tissue area can be related to the apoplastic transport of nectar substances. Different researchers mention the co-existence of both ways of nectar transport in the nectaries of various plants (Rachmilevitz and Fahn 1975; Fahn and Benouaiche 1979).

CONCLUSSION

The occurrence of the active endoplasmic reticulum, mitochondria and Golgi bodies characterise the granulocrine mechanism of nectar secretion (Rachmilevitz and Fahn 1973). Given the above statement and the fact of the occurrence of numerous vesicles and cisterns adjacent to the plasmalemma, it can be presumed that the granulocrine nectar secretion takes place in the nectaries of elecampane.

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