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# Structure of floral nectaries in *Aesculus hippocastanum* L.

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**Abstract** – Representatives of the family Sapindaceae exhibit high morphological diversity of the nectary structure. The present paper shows for the first time the results of micromorphological, anatomical, and ultrastructural analyses of floral nectaries in *Aesculus hippocastanum.* We have also described the forage and signal attractants of these flowers, which are important for the ecology of pollination. Using light, fluorescence, and electron microscopy, we demonstrated that the *A. hippocastanum* nectary forming a lobed disc is histologically differentiated into the epidermis with stomata, nectariferous parenchyma, subglandular parenchyma, and vascular bundles reaching the basal part of the nectariferous parenchyma. The use of histochemical assays revealed the presence of insoluble polysaccharides, lipids, terpenoids, and polyphenols including coumarins in the nectary tissues. Nectar is exuded onto the nectary surface via stomata and the permeable cuticle. As indicated by the observation of the ultrastructure of the nectary cells, transport of pre-nectar into parenchymal cells may proceed via the symplast and apoplast. We have also demonstrated that nectar transfer outside the protoplasts of parenchymal cells has a character of granulocrine secretion. *A. hippocastanum* flowers produce nectar abundantly; one flower secreted on average 2.64 mg of nectar and the concentration of sugars in the nectar was 33%.

**Keywords:** *Aesculus hippocastanum,* anatomy, flower, histochemical assays, micromorphology, nectar, nectary, ultrastructure

## Introduction

The genus *Aesculus* L. (Sapindaceae) comprises twelve species distributed in the northern hemisphere, eleven of them in Asia and America while one is native in Europe (Xiang et al. 1998, Forest et al. 2001). *A. hippocastanum* is a Balkan endemic and Tertiary relict, present as an autochthonous species in Bulgaria, Albania, Greece, and Macedonia (Polunin 1997, Avtzis et al. 2007). For many years, it has been widespread in nearly all parts of Europe with the exception of its northern zone (Kremer 1995). Based on morphological phylogenetic analysis, Forest et al. (2001) suggested an American origin for *Aesculus* with a single migration to Eurasia via the Bering land bridge. Harrington et al. (2005) distinguished between four subfamilies within the Sapindaceae subfamily Hippocastanoideae as well as Acereae and Hippocastaneae tribes in this subfamily.

The decorative values of *A. hippocastanum* include a regularly shaped crown, large, palmate-compound leaves, numerous white flowers forming up to 30-cm long panicles, and attractive spiky fruits (Seneta and Dolatowski 2007). Chestnut leaves, bark, flowers, and seeds are medicinal raw material containing flavonoids, coumarins, and saponins (Matysik et al. 1994, Zhang et al. 2010, Dudek-Makuch and Matławska 2013). Zygomorphic flowers of the species are polygamous (male and hermaphroditic). The perianth is formed by 4–5 petals exhibiting dimorphism and 5 fused sepals of different sizes. The generative elements comprise 5–8 stamens and one pistil with a three-loculed superior ovary. Hermaphrodite flowers are protogynous. At the time of flowering, filaments change their position. Corolla petals bear nectar guides, which change colour at the time of flowering. Flowers of this species produce nectar and pollen; therefore, they are visited by bees and bumblebees (Maurizio and Grafl 1969, Weberling 1992). Pollen is also used as food by Syrphidae (Kugler 1970). The types of trichomes producing olfactory attractants in chestnut flowers were described in our previous paper (Chwil et al. 2013).

Nectaries in the flowers of various plant species exhibit characteristic topography and structure, which constitute more or less permanent traits for genera and families (Bernardello 2007). The current knowledge of the distribution

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and structure of floral nectaries in higher taxonomic groups of orders and superorders is incomplete (Vogel 1997, Smets at al. 2000, Bernardello 2007). Simultaneously, numerous investigations reveal a high degree of anatomical diversity in terms of floral nectaries in plants (Durkee 1983). The position of the nectary within the flower may be a result of evolutionary selection taking place during the flower – pollinator interaction (Proctor et al. 1996).

The nectaries in Sapindales are receptacular (Cronquist 1981, Bernardello 2007). In the family Sapindaceae, extrastaminal annual nectaries placed between the perianth and stamens are widespread (Cronquist 1981, Cui et al. 2003). In *Koelreuteria* flowers, the nectary is situated on the slope of the androgynophore between the petal bases and the stamen whorl as a massive crested protuberance (Ronse De Craene et al. 2000, 2002). In *Cardiospermum*, the nectary is located at the base of the androgynophore and consists of two horn-like lobes. In *Urvillea*, the nectary has four lobes of different sizes (Solís and Ferrucci 2009, Zini et al. 2014), and the nectary in *Acer* forms a regular ring (Weryszko-Chmielewska and Sulborska 2011).

Maurizio and Grafl (1969) reported that the nectary in the *A. hippocastanum* flower had the shape of an irregular protuberance located between the corolla tube and stamens. Ronse De Craene et al. (2002) found that the nectary in this genus is unilaterally set between the petal bases and the stamen whorl. Acevedo-Rodrigues et al. (2011) described the nectary in *Aesculus* flowers as a 4-lobed unilateral disc.

As reported by Zini et al. (2014), currently only a few data on nectaries and their evolutionary shifts are available for most taxa. Since we found no detailed data on the structure of the *A. hippocastanum* nectary in the available literature, we undertook comprehensive studies of the morphological, anatomical, and ultrastructural traits of this nectariferous gland in order to fill the gap in this knowledge. We also used histochemical assays to reveal the content of some substances in nectary tissues that are also present in other *A. hippocastanum* organs. We have also presented the characteristics of other floral parts associated with the ecology of pollination.

## Materials and methods

The flowers were collected from 3 different trees of *Aesculus hippocastanum* in the Botanical Garden of Maria Curie–Skłodowska University in Lublin. The analyses carried out in 2014–2015 were focused on the micromorphology of the surface of the epidermis of selected floral elements as well as the anatomy and ultrastructure of the nectary. The observations were performed using stereoscopic (SM), fluorescence (FM), light (LM), scanning (SEM), and transmission electron microscopes (TEM). Floral nectaries were measured in 16 hermaphroditic and male flowers. We also analysed the quantities of nectar and sugar content in the nectar.

### Fixation of the material

Fragments of nectaries and floral elements sampled from fresh flowers on flowering day 1 were fixed in 4% glutaraldehyde for 6 h at room temperature, and next in 0.01 M phosphate buffer, pH 7.0, at 4 ºC for 48 h. After being rinsed in phosphate buffer, the samples were contrasted in a 1.5% solution of osmium tetroxide for 1.5 h. Next, the nectary fragments were dehydrated in ethyl alcohol series at concentrations of 15, 30, 50 70, 90, 96, and 99.8% for 15 min and twice in absolute alcohol. Subsequently, the material was embedded in Spurr low viscosity resin and polymerised at a temperature of 60 ºC for 48 h.

### Light microscopy

Longitudinal, semi-thin nectary sections were made from the fixed material. 0.8–1 µm sections were cut with a glass knife using a Reichert Ultracut S microtome and stained with 1% toluidine blue and 1% azure II (1:1) at a temperature of 60 ºC for 5 min.

Periodic acid Schiff (PAS) reaction was applied to localise starch grains in the plastids and other polysaccharides (cellulose and pectins) in the nectary cells (Nevalainen et al. 1972). Comparative histochemical assays, i.e. Sudan IV (Pearse 1985) and Sudan red (Brundrett et al. 1991) indicating the presence of lipid compounds in the cells as well as Nile blue A (Jensen 1962) staining acidic and neutral lipids, were carried out. We also used the Nadi-reagent (David and Carde 1964) for terpenoids, ferric trichloride for phenolic compounds, and Lugol's solution (Johansen 1940) for coumarins. Anthers were stained with Sudan red.

### Fluorescence microscopy

Hand-cut nectary sections (longitudinal) and anther samples from fresh material were placed in a droplet of a fluorochrome (0.01% auramine O) in order to analyse the cuticle layer (Wędzony 1996). The observations were carried out under a Nikon Eclipse 90i fluorescence microscope equipped with a FITC (excitation light 465–495 nm) and barrier (515–555 nm wavelength) filter.

### Scanning electron microscopy

Fixed fragments of the nectaries, corolla petals, stamens, and pistils were dehydrated in acetone series: 15, 30, 50, 70, 90, and 99.5%, and twice in absolute acetone. Next, they were critical-point dried in liquid $CO_2$ using an Emitech K850 dryer and sputter-coated with gold using an Emitech K550X sputter-coater. The surface of the epidermis of the floral elements was observed under a Tescan Vega II LMU scanning electron microscope.

### Transmission electron microscopy

Ultrathin 70-nm thick sections were cut from the fixed and resin-embedded nectaries, which were stained with an 8% solution of uranyl acetate in 0.5% acetic acid for 40 min. After double rinsing of the sections with distilled water, Reynolds' reagent was applied for 15 min (Reynolds 1963). After rinsing with water, the sections were dried. The ultrastructure of nectary epidermal and parenchymal cells in the full-secretion phase was observed under an FEI, USA Tecnai Spirit $G^2$ transmission electron microscope.

### Quantity of nectar secretion

Nectar was sampled from flowers between 9:30 and 10:30 AM with the pipette method developed by Jabłoński (2003). The sample contained nectar secreted throughout the flowers' life (within 3 days) and was collected from 6–12 flowers. Sixteen nectar samples were collected. The percent content of sugars was estimated with an Abbe refractometer (RL-1 PZO).

### Qualitative and quantitative nectar content

Quantitative and qualitative analysis of sugars in the nectar sampled throughout the flowers' life was performed with high-performance liquid chromatography (HPLC) according to Bogdanov et al. (1997) with modifications by Rybak-Chmielewska (2007).

### Statistical analyses

Standard deviation (±SD) was calculated for the measurements of the floral nectar mass, nectar sugar concentration, and nectar sugar mass using Excel 7.0 (Microsoft).

## Results

### Structure of the inflorescence and floral attractants

The number of flowers in the *A. hippocastanum* inflorescence was 155–240, with an average of 220. Among the polygamous flowers, hermaphroditic flowers (Fig. 1A), which on average accounted for 27% in the inflorescences, had a large ovary and a long style. This type of flower was found in the lower and middle part of the inflorescence. Male flowers characterised by a small, cylindrical pistil with an underdeveloped ovary (Figs. 1B, H, I) and producing no fruits represented the largest proportion (approx. 73%).

Nectar and pollen constitute forage attractants for insects in *A. hippocastanum* flowers. Signal attractants comprise yellow and red nectar guides located on petals (Figs. 1A, B), red connective protrusions on both anther poles (Figs. 1C, 2B, C), red stigmas, and odour emitted by different parts of the flower. Trichomes located on petals (Fig. 2A) as well as trichomes and colleters present on the ovary (Figs. 2G, H) may serve the function of floral attractants for insects. On the surface of the connective protrusions, we observed fluorescent secretion droplets (Figs. 1D, E). Treatment of these tissues with Sudan IV revealed the presence of lipid compounds in epidermal cells (Fig. 1F). Similarly, SEM images indicated the presence of the secretion under the cuticle convexities of the epidermal cells of these protrusions (Fig. 2 D). The stigma covered by papillae occupied a small part of the apical zone of the pistil (Figs. 2E, F).

Nectar secretion in *A. hippocastanum* flowers commences in the bud-opening phase. Throughout its lifetime, one flower secreted on average 2.64 mg of nectar. The mean concentration of sugars in the nectar was 33%. The weight

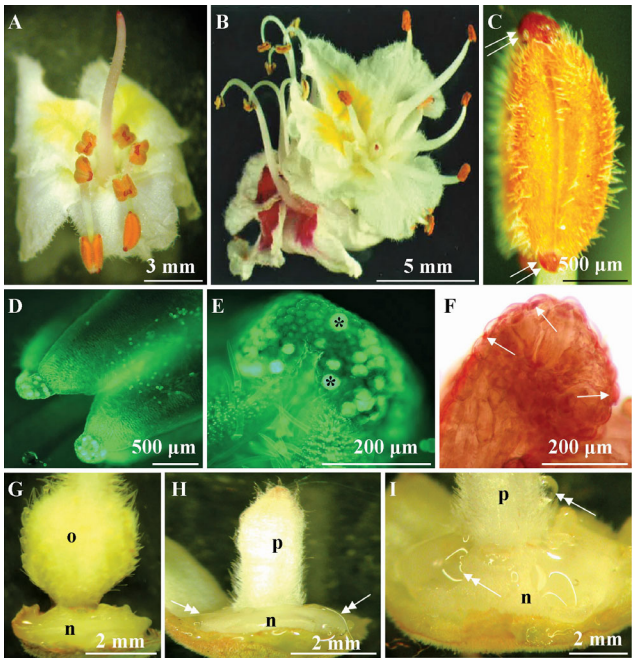


**Fig. 1.** Flowers and floral parts of *Aesculus hippocastanum*: (A, G) hermaphroditic flower; (B, H, I) male flowers with a nectary (double-headed arrow denotes nectar droplet) and a reduced pistil; (C) anther with red connective protrusions (two arrows) at the poles; (D, E) fluorescent microscopy photographs of anther parts stained by auramine O (asterisks denote fluorescent secretion); (F) longitudinal section of connective protrusion stained by Sudan red (arrows denotes lipid compounds); n – nectary, o – ovary, p – pistil.

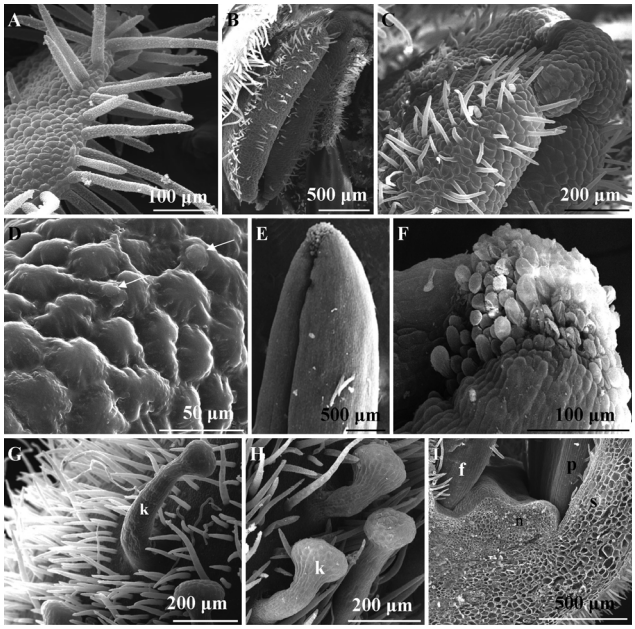


**Fig. 2.** Micromorphology of the epidermis surface of selected flower parts of the *Aesculus hippocastanum* examined by scanning electron microscope (SEM): (A) corolla petal with trichomes at the apex; (B, C) anther with protrusions at the poles and trichomes on the surface; (D) – external surface of protrusion with cuticle convexities (arrows); (E, F) – fragments of the style; papillae visible on the stigma; (G, H) trichomes and colleters on the ovary surface; (I) nectary located between the bases of filaments and portion of the petal. f – filament, k – colleters, n – nectary, p – petal, s – sepal.

of sugars was in the range of 0.31–1.39 mg per flower (Tab. 1). Sucrose, i.e. the dominant sugar in the nectar, accounted for 92% of all sugars.

**Tab. 1.** Nectar production in *Aesculus hippocastanum* flowers.

| Studied trait | min.–max. | mean±SD |
|---|---|---|
| Nectar mass (mg per flower) | 1–4.42 | 2.64±0.94 |
| Nectar sugar (percentage, %) | 25–40 | 33.05±5.04 |
| Nectar sugar mass (mg per flower) | 0.31–1.39 | 0.86±0.29 |

## Nectary micromorphology

The nectary in *A. hippocastanum* flowers, characterised by a light creamy colour, is located in the receptacle at the ovary base (Figs. 1G–I). The longitudinal section revealed a visible hollow in the central part of the nectary (Fig. 2I). The nectary gland is present in two types of polygamous flowers in this species but differs in size. It has a larger diameter in hermaphroditic flowers (on average 3.88 mm) than in functionally male flowers (on average 3.23 mm). Greater differences were noted for the nectary height in both flower types: hermaphroditic 1.01 mm, functionally male 0.61 mm.

The nectary forms an undulated ring with 4 larger and 2–3 smaller lobes. The largest lobe is adjacent to the base of two lower petals (Figs. 3A, B). On the ovary side, there are 7 concavities with the basal parts of the filaments (Figs. 3A–C). Stomata are situated in the hollow of the nectary concavities (Figs. 3D, E). At the site of the stomata, there are concavities surrounded by nonstomatal epidermal cells located above (Figs. 3E–G). Remnants of secretion were visible both on the surface of the stomata and in their vicinity. In nectary zones devoid of stomata, numerous vesicle-like cuticle convexities, probably containing secretion, and remnants of dried secretion were observed (Figs. 3H, I).

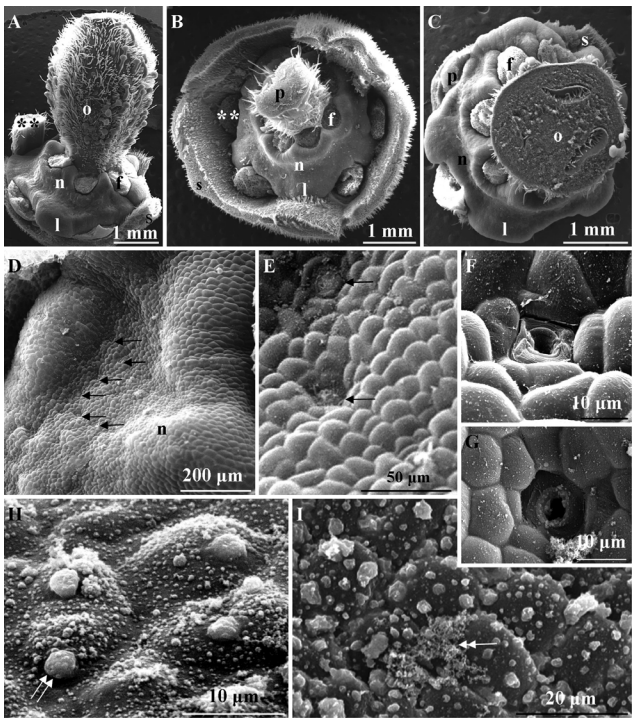


**Fig. 3.** Overall view and fragments of the *Aesculus hippocastanum* nectary examined by SEM: (A–C) lobed nectary, visible different-sized lobes; (D, E) nectary epidermis surface with stomata (arrows); (F, G) stomata between nonstomatal cells; (H, I) vesicle-like cuticle convexities in epidermal cells (double-headed arrows denote remnants of secretion); f – filament, l – lobes, n – nectary, o – ovary, p – petal, s – sepal.

## Nectary anatomy

The longitudinal nectary sections viewed under fluorescence and light microscopes showed the distribution of nectariferous cells in the central part of the nectary disc (Figs. 4A, B). The epidermis was formed by one layer of cells with relatively thin walls (Figs. 4E, G). A few stomata were observed in the sections (Fig. 4D). At the onset of the secretory activity, the cells of this tissue were already character-

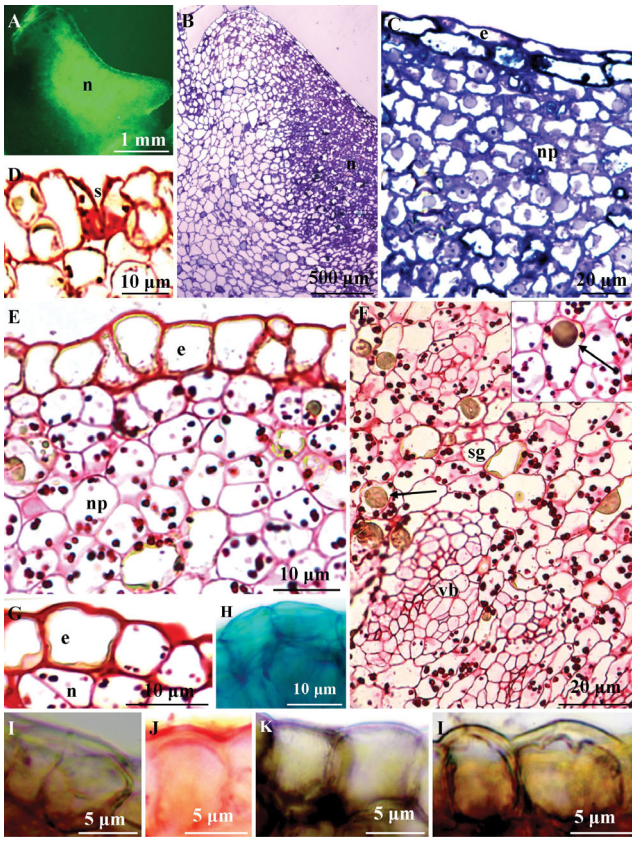


**Fig. 4.** Longitudinal sections of fragments of the *Aesculus hippocastanum* nectary observed by fluorescent microscope (A) and light microscope (B–F): (A) epidermal and nectariferous parenchymal cells; (B) parenchymal cells in the marginal and central zones of the nectary; (C) nectary epidermal and parenchymal cells; (D) stoma in the nectary epidermis; (E–G) thick-walled epidermal cells and thin-walled parenchymal cells, visible plastids with starch grains and vascular bundles (F); (H) acidic lipids stained blue with Nile blue A; (I) positive reaction of coumarins (purple staining) with Lugol's solution; (J) total lipids (red staining with Sudan IV); (K) purple-stained (Nadi-reagent) terpenes in epidermal cells; (L) phenolic compounds stained with ferric trichloride; e – epidermis, np – nectary parenchyma, sg – subglandular parenchyma, s – stoma, vb – vascular bundle; arrow in (F) denotes granular structure in the vacuole.

ised by substantial vacuolation. Small-sized parenchymal cells of the nectary were mainly located in the concave part of the nectary ring. The marginal zones of this gland were formed by considerably larger parenchymal cells (Fig. 4B). In their shape and size, the thin-walled parenchymal cells of the nectary resembled meristematic tissue. In the observed activity phase, they were characterised by a high degree of vacuolation (Figs. 4C, E). Numerous branches of vascular bundles containing phloem elements were located primarily in the subglandular parenchyma layer and some of them reached the basal part of the nectary parenchyma (Figs. 4B, F).

### Histochemistry

The histochemical assays used in the study revealed the presence of different substances in the nectary epidermis and parenchyma. Starch was detected in the nectariferous and subglandular parenchyma and in some epidermal cells with the use of the PAS-reagent (Figs. 4D–G). The treatment of nectary tissues with this reagent yielded pink staining of the cell walls in the parenchyma and vascular bundles and red staining of the epidermal cell walls. Moreover, after treatment with the PAS-reagent, parenchymal cells exhibited light brown staining of an unknown substance contained in the vacuoles and forming granular clusters in many cells (Fig. 4F).

The content of epidermal and parenchymal cells stained positively for acidic lipids with Nile blue A (Fig. 4H). Sudan IV revealed lipids in the cell wall and cytoplasm of epidermal cells (Fig. 4J). Nadi-reagent stained terpenoids purple in the same parts of these cells (Fig. 4K) and ferric trichloride gave positive reaction for phenolic compounds in epidermal cells (Fig. 4L). Lugol reagent revealed coumarins stained purple and bright red in epidermal cells (Fig. 4I, Tab. 2).

### Nectary ultrastructure

The epidermis cells had various shapes and overlapped at some sites. The outer wall was thicker than the radial walls and the inner tangential wall. The central part of the cell was occupied by a large vacuole (Fig. 5A) or a central cytoplasm band containing one large cell nucleus, numerous mitochondria, plastids, and ER separating smaller vacuoles. The vacuoles of some nectary epidermal and parenchymal cells contained deposits of an osmophilic substance resembling deposits observed in the epidermal and parenchymal cell of the calyx (Figs. 5A, H).

Small intercellular spaces were observed in nectary parenchyma (Figs. 5A–C). Numerous plasmodesmata were present in the cell walls (Fig. 5D). A substantial part of the protoplast was occupied by cell nuclei. A great number of mitochondria mainly characterised by tubular arrangement of inner membranes were observed in the electron-dense

**Tab. 2.** Chemical compounds detected with histochemical assays in the epidermal cells in *Aesculus hippocastanum* nectar. PAS – periodic acid Schiff.

| Histochemical assay | Compound group | Cell staining | Nectary epidermal cells |
|---|---|---|---|
| Sudan IV | fats | red | ++ |
| Sudan red | lipids, resins, oils, waxes | red | ++ |
| Lugol reagent | coumarins aesculin | grey-purple red | + |
| Nile blue A | acidic lipids | blue | ++ |
| | neutral lipids | pink | – |
| Nadi-reagent | terpenoids | purple-blue | ++ |
| Ferric trichloride | polyphenols | black | + |
| PAS-reagent | polysaccharides | red | + |
| Auramine O | cutin, suberin | green | ++ |

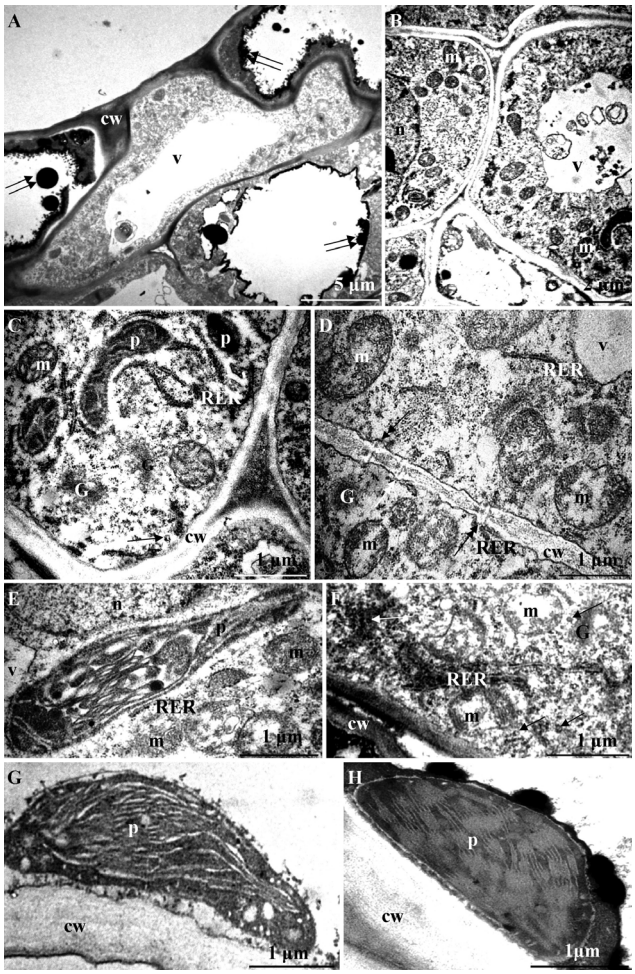


**Fig. 5.** Ultrastructure of *Aesculus hippocastanum* nectary cells examined by transmission electron microscope: (A) epidermal and subepidermal cells (two arrows denote deposits of an osmophilic substance); (B) parenchymal cells with a centrally located nucleus and vacuoles of different size, electron-dense cytoplasm, numerous mitochondria, plastids, membranous structures in the vacuole; (C) cytoplasm with polymorphic plastids, rough endoplasmic reticulum (RER), Golgi apparatus, and transport vesicles (arrow) at the plasmalemma; (D) cell wall with plasmodesmata (double-headed arrows), plasmalemma invaginations, numerous parietal situated mitochondria, RER profiles; (E, G) polymorphic plastids with varied arrangement of inner membranes; (F) well-developed RER, numerous vesicles (arrows), mitochondria, and Golgi apparatus; (H) plastid from the cell of sepal; cw – cell wall, G – Golgi apparatus, n – nucleus, p – plastid, v – vacuole, m – mitochondrion.

cytoplasm (Figs. 5C, D). Polymorphic plastids exhibited an electron-dense stroma and irregular arrangement of the system of internal membranes with bright content and plastoglobules therein (Figs. 5E, G). The structure of the nectary plastids was markedly different from the structure of chloroplasts with regularly distributed grana thylakoids originating from the parenchyma of the sepals (Fig. 5D). The cytoplasm exhibited numerous profiles of rough ER forming characteristic configurations around the plastids and in the peripheral parts of the cytoplasm (Figs. 5C, F). Furthermore, we observed Golgi apparatuses and numerous ribosomes. Some intercellular spaces contained a grey substance with vesicle-like structures (Fig. 5C). Numerous vesicles formed clusters near the plasmalemma (Figs. 5C, F).

## Discussion

High morphological diversity of nectaries can be found within the family Sapindaceae. A large (3–4 mm in diameter), lobed nectary is characteristic for *A. hippocastanum*. Our investigations partly confirmed the findings reported by Ronse De Craene (2002) and Acevedo-Rodríguez et al. (2011), who described the nectaries of the analysed species as lobed, unilateral discs. Zygomorphic *A. hippocastanum* flowers exhibit bilateral symmetry of the nectary. The best-developed lobe of this gland is directed towards petals that have the lowest location. The flowers in this species are horizontally arranged in the inflorescence. Abundant nectar accumulates in the lower part of the flower and numerous trichomes located on the petals, sepals, and ovary probably protect it against outflowing.

Dafni and Kevan (1996) showed that bilaterally symmetrical nectar guides were a characteristic trait of zygomorphic flowers. This trait was found in the *A. hippocastanum* flowers as well. In terms of location in the flower and morphological traits, it can be noted that there is certain similarity of the *A. hippocastanum* nectary to the undulated nectary disc in *Koelreuteria*, a genus from the family Sapindaceae presented in the paper of Ronse De Craene et al. (2000).

As indicated by various authors, nectaries with bilateral symmetry are also present in plant species from other families producing zygomorphic flowers, e.g. in Lamiaceae (Dafni et al. 1988, Weryszko-Chmielewska 2000) and in Ericaceae from the genus *Rhododendron* (Weryszko-Chmielewska and Chwil 2005, 2007).

Various authors report that nectar in Sapindaceae is exuded onto the nectary surface via stomata, as shown in *A. hippocastanum* in this study. Nectarostomata have been observed in floral nectaries in *Acer platanoides* (Weryszko-Chmielewska and Sulborska 2011), three *Cardiospermum* species, and *Urvillea* (Solís and Ferruci 2009, Zini et al. 2014). In contrast, Ronse De Craene et al. (2000) did not find stomata in the nectary of *Koelreuteria.* Our study indicates that nectar in *A. hippocastanum* can be secreted via not only stomata but also the permeable cuticle of the nectary, which forms a thin layer and covers very numerous vesicles, probably containing the secretion. The possibility of existence of two pathways of nectar exudation onto the nectary surface is confirmed by data provided for other plant species, e.g. *Prunus* (Radice and Galati 2003) and *Citrus* (Konarska and Weryszko-Chmielewska unpublished data).

*A. hippocastanum* flowers are characterised by abundant nectar secretion. In our paper, we have shown that the weight of nectar secreted by one chestnut flower was in the range of 1–4.42 mg, with an average of 2.64 mg. These values are close to those reported by Maurizio and Grafl (1969) for this species. Our results concerning the sugar content in the nectar (25–40%) differ from the findings shown by Maurizio and Grafl (1969), i.e. 60–76%. In our study, sucrose was the dominant sugar in the nectar of the analysed species, likewise in the earlier data provided by different authors (Maurizio 1959, Maurizio and Grafl 1969, Fahn 1979).

A relationship between the type of nectar, form of the nectary, and type of visitors was noted. Nectar with high sucrose content is used by various pollinator insects, e.g. moths, butterflies, and long-tongued bees. The sugar content in nectar collected by bees is approximately 40% (Proctor et al. 1996, Nicolson et al. 2007). Besides, zygomorphy and yellow and blue or purple flower colours belong among pollination signals for bees (Proctor et al. 1996). Since several of these traits are exhibited by *A. hippocastanum* flowers, the plants can be regarded as adjusted to bee pollination. Both anther poles bear red appendages, which not only play a role of visual attractants for insects but also may provide them with food, as they release lipid compounds.

Our investigations indicate that some epidermal cells of the *A. hippocastanum* nectary produce phenolic compounds (including coumarins), which may protect the gland against being consumed by insects. As shown in the literature, phenolic compounds serve an important protective function by exerting an adverse effect on the biology of herbivorous insects (Oleszek et al. 2001). Cisowski (1983) demonstrated the presence of aesculin, i.e. a coumarin compound, in *A. hippocastanum* flowers. We additionally detected terpenoids (Nadi-reagent) in the *A. hippocastanum* nectary. A positive reaction of the *Ananas ananassoides* nectary to this reagent was reported by Stahl et al. (2012). The results of these assays may indicate the presence of essential oils in the analysed nectaries.

At the onset of nectar secretion, we found low numbers of starch grains in the plastids from the nectary parenchyma cells in the semithin sections. In ultrastructural analyses of the nectary, we did not demonstrate starch content in these cells, which must have been related to the more advanced phase of nectary activity. The plastids observed in our study on electronograms were characterised by a system of inner membranes similar to those present in tubular chromoplasts demonstrated by Woźny et al. (2001) and Evert (2006).

Based on the ultrastructural traits of the *A. hippocastanum* nectary, it is possible to define the mode of nectar transfer outside the protoplast in the cells of the nectariferous parenchyma. The great number of mitochondria, numerous RER profiles, Golgi structures, and very abundant vesicles located in close proximity to the plasmalemma and in the cell wall may indicate a granulocrine mode of nectar secretion. Granulocrine secretion has been demonstrated by authors of other studies on the nectary structure in a number of plant species (Fahn and Benouaiche 1979, Sawidis 1989,

O'Brien et al. 1996, Weryszko-Chmielewska et al. 2006, Wist and Davis 2006, Chwil and Chwil 2012, Kowalkowska et al. 2015).

The numerous plasmodesmata present in the parenchymal cell walls in the *A. hippocastanum* nectary indicate symplastic transport of nectar, whereas the presence of the secretion in the intercellular spaces and transfer thereof to the stomata suggest a possibility of nectar transport via the apoplast. Two pathways of nectar transport within nectariferous tissues have been shown by Radice and Galati (2003) in *Prunus* as well as Chwil and Chwil (2012) in *Polemonium*.

Many authors believe that the location and structure of floral nectaries are important plant taxonomic trait (Rudall et al. 2000, Smets et al. 2000, Bernardello 2007, Chwil and Weryszko-Chmielewska 2012). In the present study, we presented for the first time the micromorphology, anatomy, and ultrastructure of the *A. hippocastanum* nectary, which expands the knowledge of the nectary structure and may complete the set of diagnostic traits used in taxonomy. Our research resulted in following main conclusions: 1. A majority of flowers (ca. 73%) in *A. hippocastanum* inflorescences are functionally male flowers, which produce an underdeveloped ovary. The other part is represented by hermaphroditic flowers. 2. *A. hippocastanum* flowers offer pollinators various food attractants: abundantly secreted nectar, pollen, and colourful food bodies located on both anther poles. 3. Nectaries are present in both types of *A. hippocastanum* flowers; despite the different sizes, they have the same structure. Nectar exudation onto the nectary surface proceeds in two ways: via stomata and the permeable cuticle. Nectary cells contain phenolic compounds and emit aromatic substances.

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