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THE COMPARISON OF NECTARIES STRUCTURE OF SOME VARIETIES OF ORNAMENTAL APPLE

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S u m m a r y

The study of floral nectary structures of thirteen orna mental apple cultivars examined using light microscope (MS) and scanning electron microscope (SEM) was performed. It was found that nectary glands in the selected cultivars were located in the upper part of the flower receptacle, between the ovary of the pistil and the base of stamen filaments, and they generally belonged to the epimorphic or transitoric type. The nectary sur face area, its thickness, the number of glandular tissue layers, the height of epidermal cells of the nectary and the thickness of the outer wall of the epidermis, together with the cuticle, were de termined by light microscope. By using SEM, the structure of the surface of nectaries in four ornamental apple cultivars was observed. The epidermis of the upper part of the nectaries was composed of elongated cells of which outer cell wall was cove red with a striated cuticle. The remaining part of the nectary was characterised by cells of similar arrangement and shape, but their surface was marked by a thinner and smoother layer of cuticle. Closed or opened stomata were generally situated at the level of the epidermal cells. Their pores were often filled with granular or plate shaped structures.

Key words: nectaries, structure, ornamental apple

INTRODUCTION

Ornamental apple species growing in Poland mainly come from Asia and Europe. They are used in different types of plantings, primarily in parks and large green areas; they are also popular in Japanese type gardens. They are most often planted due to their attractive white, pink or pink-purple flowers (S z w e y k o w s c y, 2003; S e n e t a and D o l a t o w s k i, 2004). Yellow, yellow-green, red or orange-red fruits of these cultivars are also very interesting. Due to their flowering period (May) and the abundance of flowers produced, ornamental apple trees can be regarded, likewise cultivated apple trees, as a good early spring source of nectar and pollen for pollinating insects.

Earlier studies on various apple cultivars show that the percentage contribution of sugars in nectar of these plants ranges between 23 and 29% (G u l y á s et al., 1989). Sucrose is the predominant sugar in their nectar, whereas glucose and fructose occur in them in smaller amounts (O r o s z - K o v á c s et al., 1997). According to many researchers, the amount of nectar secreted and the concentration of sugars in it, the main factors affecting the attractiveness of nectar to pollinators, are chiefly determined by the size and thickness of nectary glands, as well as their structure (O r o s z - K o v á c s et al., 1990; D a v i s and G u n n i n g, 1992; W e r y s z k o - C h m i e l e w s k a et al., 1997).

In this study, the aim was to determine the size and several anatomical parameters of floral nectaries in thirteen ornamental apple cultivars and to compare the surface structure of these glands in four selected cultivars.

MATERIAL AND METHODS

Flowers of the following cultivated and botanical cultivars of ornamental apple were sampled: *Malus baccata* var. *jackii*, *M. sieboldi* var. *arborescens*, *M. floribunda* `Van Houtte`, *M. x zumi* `Szafer`, *M. x zumi* `Hopa`, *M. x zumi* `John Downie`, *M. x zumi* `Calocarpa`, *M. x zumi* `Wintergold`, *M. x zumi* `Gorgeous`, *M. x zumi* `Henrietta Crosby`, *M. x zumi* `Lady Northcliffe`, *M x zumi* `E.H. Wilson`, *M. x zumi* ` Sikora B`. The plants came from plantings in the Botanical Garden of the Maria Skłodowska-Curie University in Lublin, Poland. Flowers were collected on the second day after petal opening, at the beginning of pollination.

The flowers, after the removal of the perianth and stamens, were analysed using light and scanning electron microscopies. For morphometric examination by light microscope, the material was fixed in 70% alcohol and 4% glutar aldehyde. Longitudinal sections were

made using the Vibratome 2000 (Technical Product International, INC, USA). About 60 μm – thick sections were embedded in glycerol-gelatine.

The following features were analysed by light microscopy in slides coming from five flowers of each taxon: the nectary type, the surface area of the nectary, the thickness of the nectary measured at its half-length, the number of layers of glandular cells, the height of the nectary epidermal cells and the thickness of the outer wall of the epidermis, together with the cuticle. The nectary surface area was assumed to be the lateral area of a truncated cone determined by the diameter of the flower in the lower and upper parts of the nectary and by its length, which were calculated in accordance with the following formula:

$$P = \Pi\ (R+r)\ l$$

$$l = \sqrt{h^2 + (R-r)^2}$$

$$P = \Pi(R + r)\ \sqrt{h^2 + (R-r)^2}$$

(symbols are described in Fig. 1).

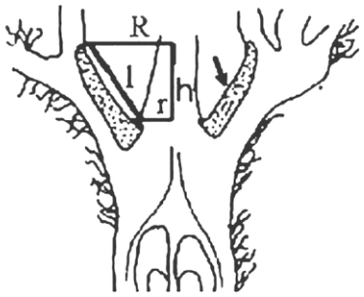


Fig. 1. Diagram of longitudinal section of the lower part of flower of the ornamental *Malus* with marked nectaries (arrow.).

To observe surface of the gland nectaries by scanning electron microscope, fragments of flowers were sampled from four ornamental apple cultivars, i.e. *M. baccata* var. *jackii*, *M. sieboldi* var. *arborescens*, *M. x zumi* `Hopa` i *M x zumi* `John Downie`. The material was fixed in 4% glutar aldehyde in 0.1M phosphate buffer with the pH of 7.2. Next, the samples were dehydrated in acetone series and dried at critical point, then coated with gold using the CS 100 Sputter coater. Observations were made in the BS – 300 Tesla scanning microscope.

RESULTS

The gland nectary in flowers of the studied ornamental apple cultivars is located on the adaxial surface of the flower receptacle, between the upper part of the ovary of the pistil and the base of stamens, and it forms a thick layer. As it is composed of receptacle tissues, it was included in receptacular nectaries (Fig. 2).

In terms of the convexity or the planar position, the nectaries of the studied botanical and cultivated ornamental apple cultivars were classified as epimorphic (planar) or transitoric (intermediate), rarely automorphic (convex) (Tab. 1, Fig. 2).

In most of studied apple taxa, the glandular tissue forms a uniformly-thick layer in the flower, except at the cultivar `Hopa` in which the lower part of the nectary bordering the base of the style was clearly widened.

Significant differences between the analysed cultivars were in the size of the nectaries were observed (Tab. 1). The nectaries of *M. x zumi* `Gorgeous` and *M. x zumi* `C*alocarpa*` had the largest surface area, 8.3 mm^2 and 7.3 mm^2, respectively. Ornamental apple *M. x zumi* `Szafer` was characterised by glands with the smallest surface area (4.17 mm^2).

In most of the cultivars studied, the number of layers of glandular cells ranged between 20 and 23. But in *M. sieboldi* var. *arborescens*, a much smaller number was observed (14 layers), whereas their largest number was in *M. x zumi* `Gorgeous` (31 layers) (Tab. 1). It is worth noting that the number of glandular tissue layers did not always show a relation with the gland's thickness. Notably, the gland nectaries in *M. x zumi* `E.H. Wilson`, with 14 layers of secretory tissue, had the thickness similar to that of the nectaries in *M. x zumi* `Sikora B`, with 29 layers of secretory tissue. The above-mentioned feature is most probably connected with the different size of glandular cells in particular cultivars. But the relation between these two features occurred in the case of the nectaries of *M. x zumi* `Gorgeous` and *M. x zumi* `E.H. Wilson` (Tab. 1).

The size of epidermal cells of the nectary in the apple cultivars studied was different. In most of the cultivars, it ranged between 10 and 14 μm, the largest being in *M. x zumi* `Henrietta Crosby` (about 18 μm), and the smallest in *M. sieboldi* var. *arborescens* (about 7.5 μm). The the outer wall of the nectary epidermal cells, together with the cuticle, showed a similar thickness (3-4 μm) in most of the analysed plants. Only in three cultivars, i.e. *M. baccata* var. *jackii*, *M. x zumi* `Szafer` and *M. x zumi `calocarpa`*, it was almost two-fold larger (5-6 μm).

SEM observations show that the selected cultivars were characterised by a similar structure of the nectary epidermis. The epidermis ofthe highest part of the nectary was composed of elongated cells with a convex outer walls which formed a specific arrangement resembling "wickerwork" (Figs. 3, 6). Their surface was covered by a cuticle layer with clearly different depth striae running parallelly to the longer axis of the epidermal cells (Figs. 4, 6, 7, 9, 13). The remaining part of the nectary had cells of similar arrangement and shape, but their surface was marked by a thinner and smooth cuticle (Figs. 5, 8, 10, 14). The stomata, through which nectar

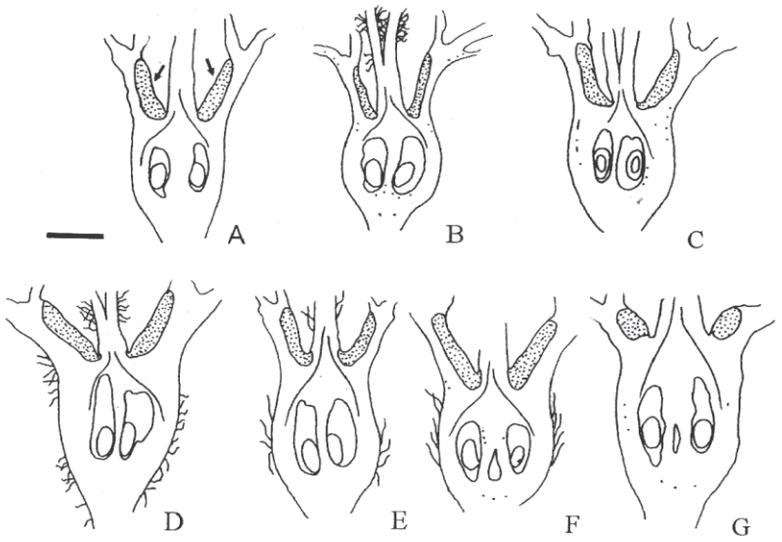
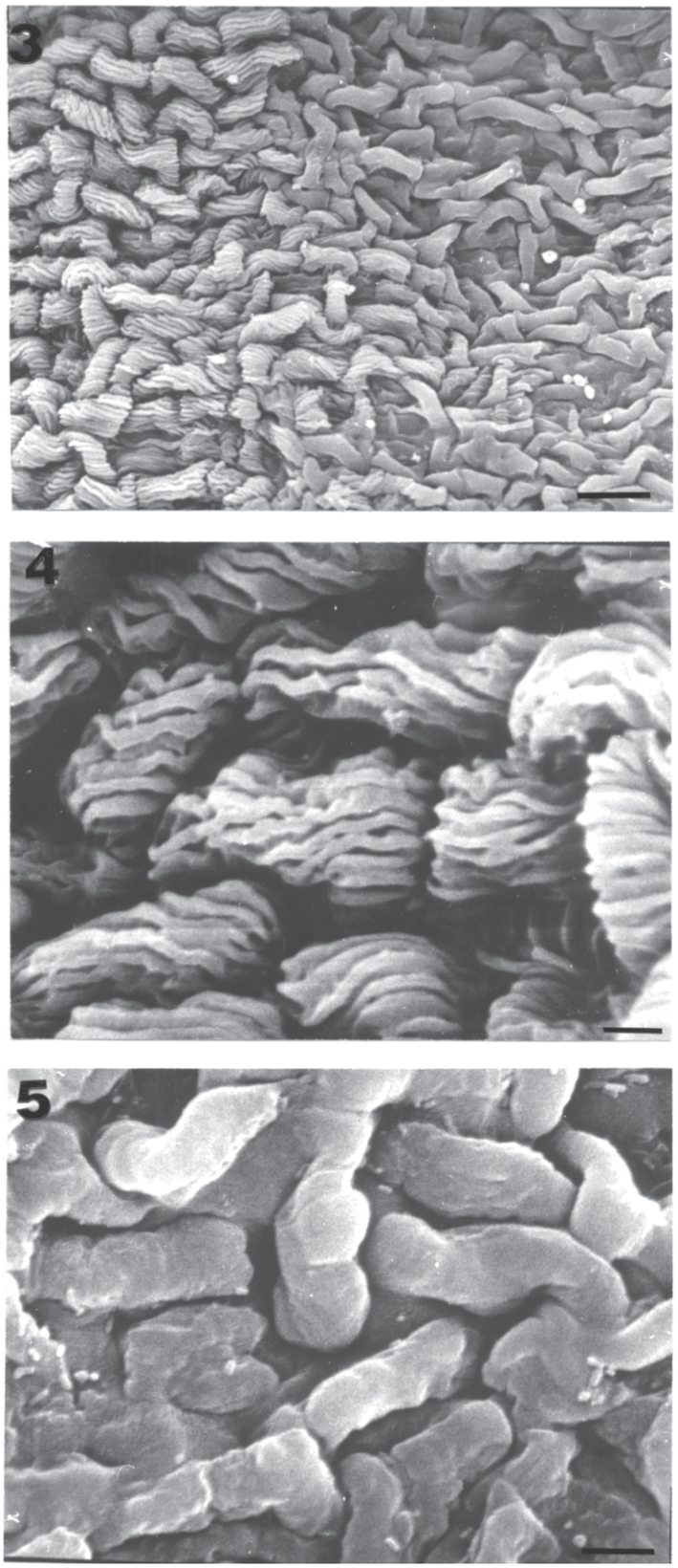


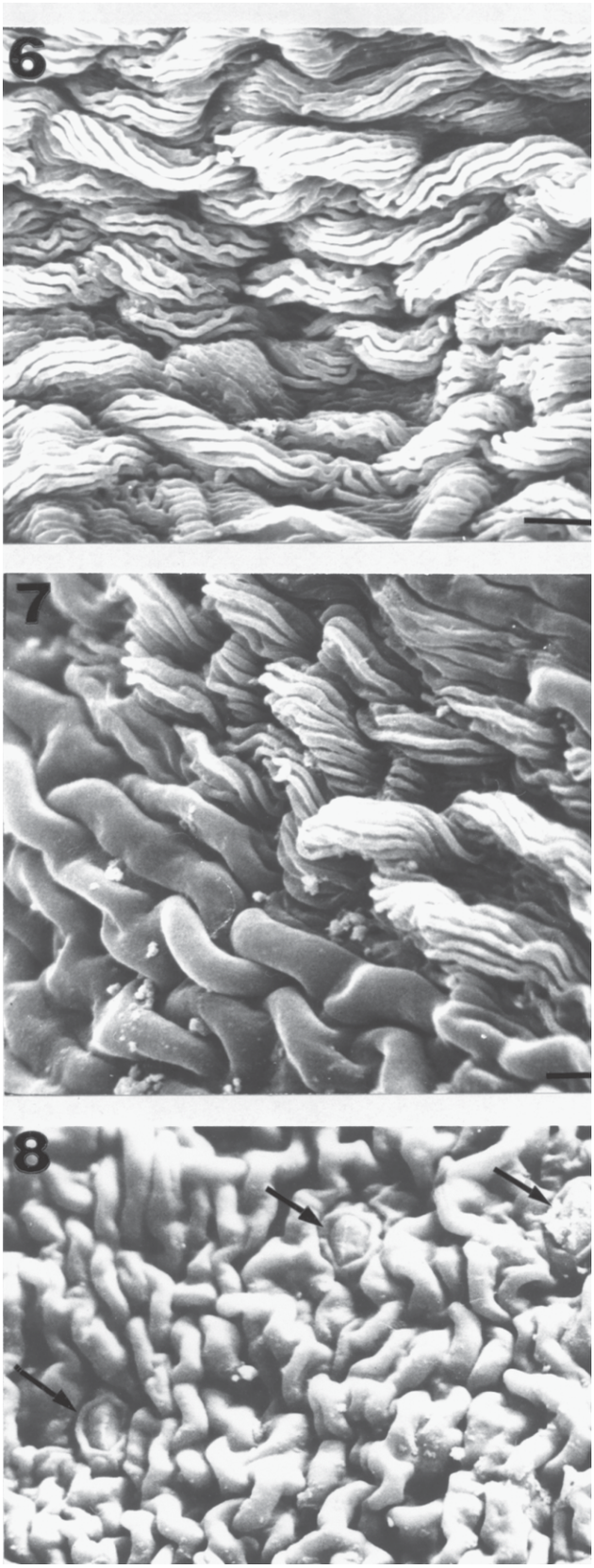
Fig. 2. Diagrams of longitudinal sections of the lower part of flowers of the studied apple cultivars with marked nectaries (arrows): A *Malus baccata* var *jackii*, B *M. sieboldi* var. *arborescens,* C *M. x zumi* `Szafer`, D M. x zumi `Jon Downie`, E *M. x zumi* `Henrietta Crosby`**,** F *M. x zumi* `Lady Northcliffe`, G *M. x zumi* `Sikora B`. Bar 2 mm.

Table 1
Results of cross section surface measurement nectaries in the studied ornamental apple cultivars.

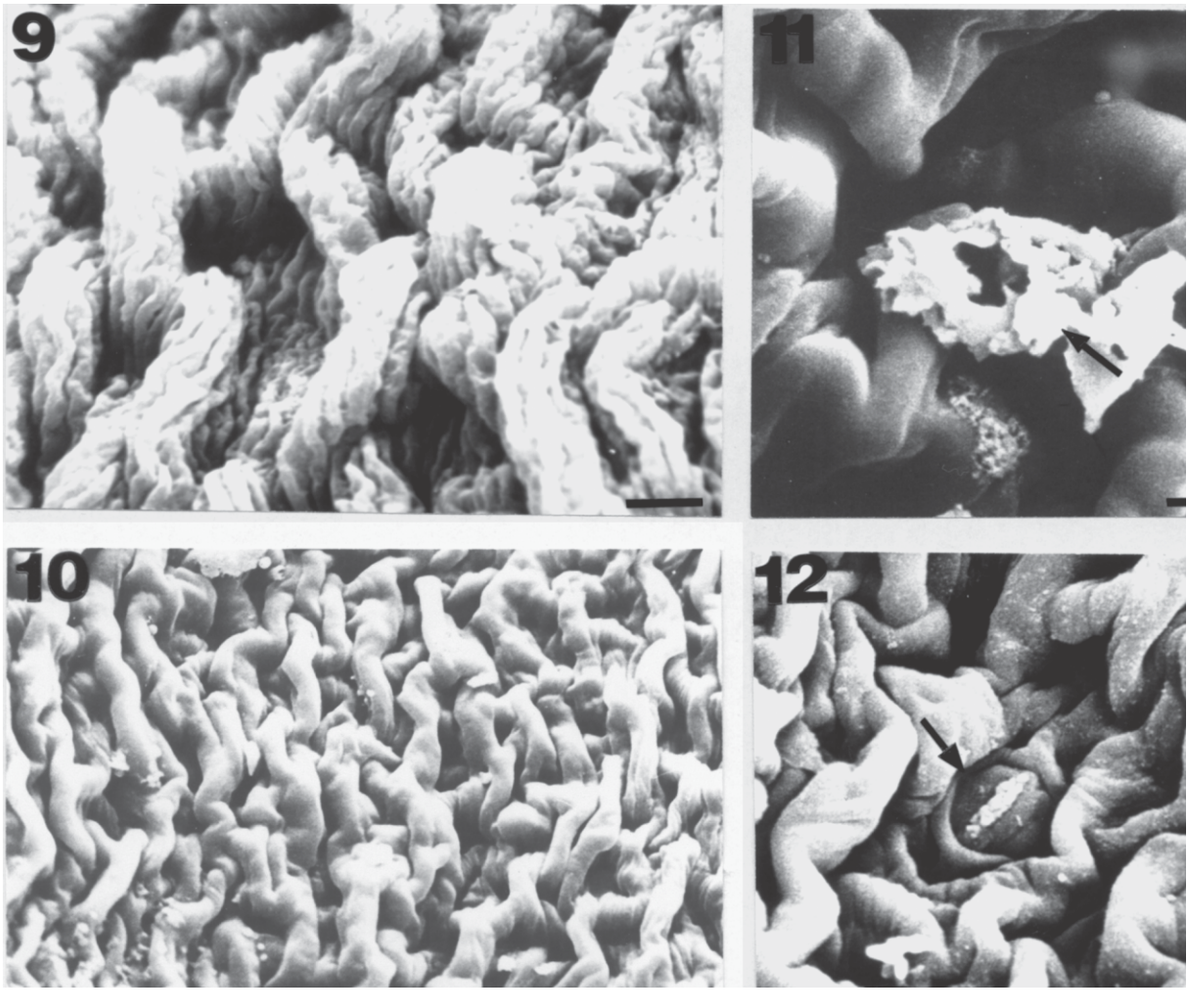
Taxon	Nectary type	Surface of nectary ($mm^{2)}$	Number of layers of grandular cells	Height of nectary epidermal cells (µm)	Thickness of outer wall of epidermis with cuticle (µm)	Thickness of gland layer (µm)
Malus baccata var. jackii	transitoric	4.67	18	10.26	5.57	366.7
M. sieboldi var. arborescens	epimorphic	4.51	14	7.60	3.78	205.0
M. floribunda `Van Houtte`	transitoric	4.41	17	10.50	3.33	210.0
M. x zumi `Szafer`	epimorphic	4.17	23	10.15	6.09	357.5
M. x zumi `Hopa`	epimorphic	5.79	21	10.50	3.61	282.0
M.x zumi `Wintergold`	transitoric	5.28	22	12.25	3.99	302.5
M. x zumi `John Downie`	epimorphic	5.89	20	9.10	3.62	390.0
M. x zumi `Calocarpa`	epimorfic	7.31	21	10.85	5.32	202.5
M. x zumi `Gorgeous`	automorphic or epimorphic	8.32	31	12.15	3.22	455.0
M x zumi `Henrietta Crosby`	epimorphic	5.08	21	28.43	3.15	262.5
M. x zumi `Lady Northcliffe`	epimorphic or transitoric	6.51	28	14.18	3.43	387.5
M. x zumi `E. H. Wilson`	epimorphic or transitoric	6.58	21	13.20	2.45	410.0
M. x zumi `Sikora B`	automorphic	6.00	29	11.66	3.54	430.0



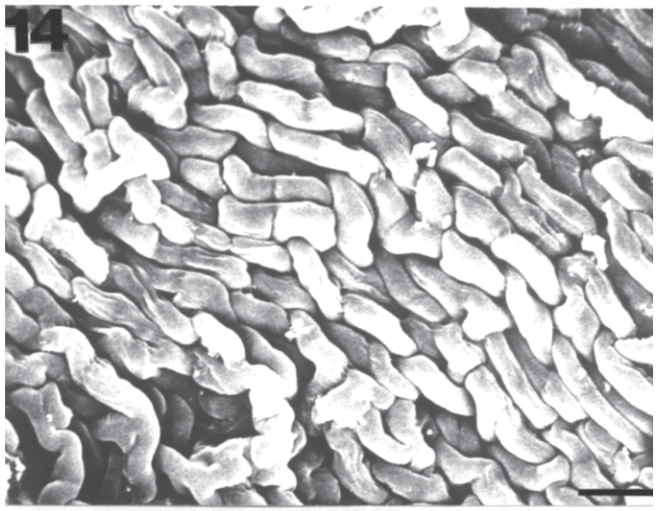
Figs. 3 5. Fragments of nectary surface of *Malus baccata var. jackii* with striated (3, 4) and smooth (5) cuticle. Bar 20 µm (3), bars 5 µm (4, 5).

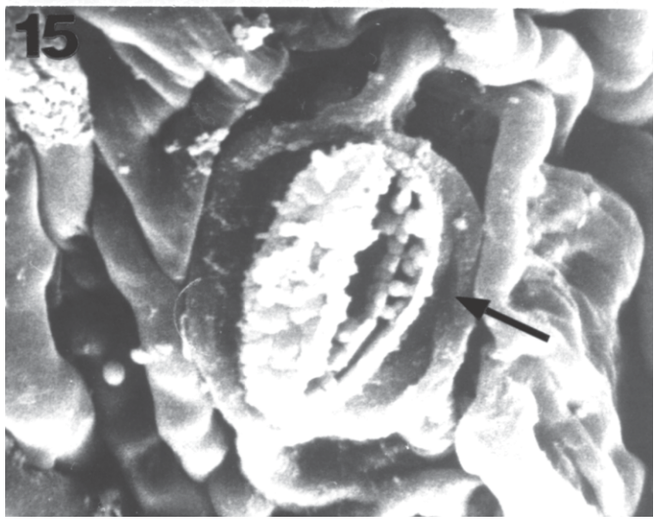


Figs. 6 8. Fragments of surface nectary of *Malus sieboldi* var. *arborescens* with striated (6, 7) and smooth (7, 8) cuticle and with stomata (arrows) (8). Bars 10 μm.



Figs. 7 10. Fragments of nactary surface of *Malus x zumi* `Hopa` with striated (9) and smooth (10) cuticle and with stomata (arrows) (11, 12). Bars 10 µm (9, 11, 12), bar 20 µm (10).





Figs. 13 15. Fragments of nectary surface of *Malus x zumi* `John Downie` with striated (13) and smooth (14) cuticle and with stoma (15) (arrow). Bars 5 µm (13, 15), bar 10 µm (14).

is secreted, were located only in the part of the nectary with a smooth cuticle without striae. The stomata were few, closed or open, and situated at the level of the epidermal cells or in small hollows (Figs 8, 11, 12, 15). At places, dried nectar concentrated around the stomata as lobes (Fig 11), as well as its different-sized spherical forms (Fig 15), were also observed.

DISCUSSION

The investigations show that the location and type of nectaries of ornamental apple are characteristic for the genus *Malus* and for many other taxa of the subfamily *Pomoideae* (Weryszko-Chmielewska and Konarska, 1995; Chwil et al., 2006; Konarska, 2006; Weryszko-Chmielewska and Konarska, 2006). It is difficult to compare the surface sizes of the nectaries of ornamental cultivars obtained in this study to the size of nectaries in other genera from *Pomoideae*, or even to cultivated cultivars, due to different methods of gland surface measurements. However, the author's earlier studies relating to nectaries in *Malus silvestris* show that the thickness of the nectary in this species is smaller than the thickness of this gland in the majority of ornamental cultivars (Konarska, 2006). The literature list shows that not only the size of the nectary, but also the thickness of this gland, affect the amount of nectar produced as well, what is connected with the number of nectar-secreting cells (Orosz-Kovács et al., 1990).

I found the occurrence of stomata in the nectary epidermis, probably being the only way of nectar secretion. However, no convexities or breaks in the cuticle, which had been observed by Orosz-Kovács et al. (1990) in nectaries of cultivated cultivar, were observed in this study. They could be other way of nectar secretion in apple flowers. Numerous papers describe the presence of modified (permanently open) stomata in the nectariferous tissue of many *Pomoideae* representatives (Weryszko-Chmielewska et al., 2003; Konarska et al., 2005; Konarska, 2006). Both open and closed stomata, observed in this study, could suggest that they perform rhythmic movements, as well as they can evidence a controlled outflow of nectar. Nagy Toth et al. (2000) made similar observations in cultivated apple cultivars. Moreover, according to these authors, the location of stomata in hollows or at the level of epidermal cells couldbe related to the adaptation of this species to its environment. The position of the stomata mainly at the level of the gland epidermal cells would indicate the occurrence of mesomorphic features in the studied cultivars.

The nectaries of the studied apple cultivars were covered in their upper part by a quite thick layer of cuticle with very characteristic striation. The thickness of the outer wall of the nectary epidermis in these cultivars was much larger than in a related species of *M. silvestris* (Weryszko-Chmielewska and Konarska, 1995). As it is known, the cuticle with clear and deep ornamentation favours the longer retention of nectar on the surface of the nectary and protects secretion against drying (Orosz –Kovács, 1990; Nagy Toth et al., 2000). In addition, the cuticle containing wax on its surface protects against excessive solar radiation (Juniper and Cox, 1973; Hejnowicz, 2002), what can also favour the longer maintenance of nectar in liquid state on the surface of an exposed and entirely unprotected nectary gland.

On the basis of the obtained results relating to the structure of floral nectaries, it can be stated that the analysed ornamental apple cultivars, can be a valuable early spring benefit for pollinating insects. In order to determine exactly the usefulness of these cultivars, it is necessary to investigate the amount of produced nectar, what will be studied in the neaest future.

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Porównanie struktury nektarników wybranych odmian jabłoni ozdobnych

Streszczenie

Badano dotyczyły struktury nektarników kwiatowych kilkunastu odmian jabłoni ozdobnych, przy zastosowaniu mikroskopii świetlnej (MS) oraz skaningowej elektronowej (SEM). Stwierdzono, że gruczoły nektarnikowe wybranych odmian położone były w górnej części dna kwiatowego, między zalążnią słupka, a podstawą nitek pręcikowych i należały z reguły do typu epimorficznego lub tranzytorycznego. W mikroskopie świetlnym określono pole powierzchni nektarnika, jego miąższość (grubość), liczbę warstw tkanki gruczołowej, wysokość komórek epidermy nektarnika oraz grubość zewnętrznej ściany epidermy łącznie z kutykulą. W SEM obserwowano strukturę powierzchni nektarników czterech odmian jabłoni ozdobnych. Epiderma górnej części nektarników zbudowana była z wydłużonych komórek, których zewnętrzna ściana komórkowa pokryta była prążkowaną kutykulą. Pozostała część nektarnika charakteryzowała się komórkami o podobnym ułożeniu i kształcie, ale ich powierzchnia odznaczała się cieńszą i gładką warstwą kutykuli. Zamknięte lub otwarte aparaty szparkowe leżały z reguły na poziomie komórek skórki. Ich pory często wypełnione były ziarnistymi lub płytkowatymi strukturami.