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MORPHOLOGY OF NECTARIES IN RELATION TO NECTAR SECRETION OF *INULA* AND *CENTAUREA* SPECIES IN THE HUNGARIAN HABITATS

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Floral nectary characteristics of five species of *Centaurea* and *Inula* (*Inula ensifolia*, *I. salicina*, *I. spiraeifolia*, *Centaurea micranthos*, *C. scabiosa*) were examined for using light microscopy and scanning electron microscopy, and the sugar contents were studied in relation to nectary morphology and plant size. The differences in the nectaries, plant size and nectar sugar content of the studied species indicate that the larger plants may reflect a greater allocation to sugar content.

Key words: floral nectary, glandular tissue, plant size, size and form of nectary, sugar content of nectar

INTRODUCTION

Asteraceae is one of the richest-in-species families of the plant kingdom. In spite of its big size we have only a few knowledge about the nectaries of its species, and we can find no articles concerning on the size and the nectar production of their nectaries.

Several articles were born for ultrastructure of nectaries (Fahn 1979), and nectar chemistry (Percival 1961, Baker and Baker 1990, Carlisle and Ryle 1955). The plant species nectar is the principal floral reward (Simpson and Neff 1983). Both the quantity and quality of floral nectar vary among species, and this variation is correlated with pollinator type (Baker and Baker 1975, 1983, Cruden *et al.* 1983). The characters of the nectar have been correlated with plant characters such as sexual stage, position in an inflorescence, flower age, plant size, and flower size (Rathcke 1992). Nectar production has been shown to be influenced by environmental factors such as time of day (Hodges 1993, Pleasants 1983, Marden 1984), water availability (Pleasants 1983, Zimmerman 1983), and humidity (Corbet *et al*. 1979, Bertsch 1983) and also by developmental factors such as the flower position in the inflorescence (Percival and Morgan 1965, Pyke 1978, Devlin *et al.* 1987), and flower age (Pyke 1978, Schemske 1980, Pleasants 1983, Devlin *et al.* 1987).

The positions, form and size of nectaries of five Asteraceae species were determined, and the correlation between the nectar production (sugar content) and nectary size, nectary structure, and plant size was also studied.

MATERIALS AND METHODS

Study sites

The species were chosen based on their appearance in their natural habitats. Inflorescences of the investigated species were collected in July 1997 at the following localities:

1. South Hungary: Mecsek Mts, on Tettye Hill, in typical karstic shrub-forest on the southern slope, with forest-steppe and steppe elements such as *Inula spiraeifolia* L.

2. North Hungary: in the Aggtelek-karst area, where the natural succession resulted here in semi-dry grassland. Dominant species in this area, e.g. *Centaurea scabiosa* L., *Coronilla varia* L., *Dorycnium germanicum* (Gremli) Rikli, *Inula ensifolia* L., *Inula salicina* L., *Salvia verticillata* L. and *Teucrium chamaedrys* L., with the strating bordering, rimming, *Brachypodium pinnatum* (L.) P. B., *Carex montana* L. and sprouting dicotyledons covering as much as 50% in patchwork.

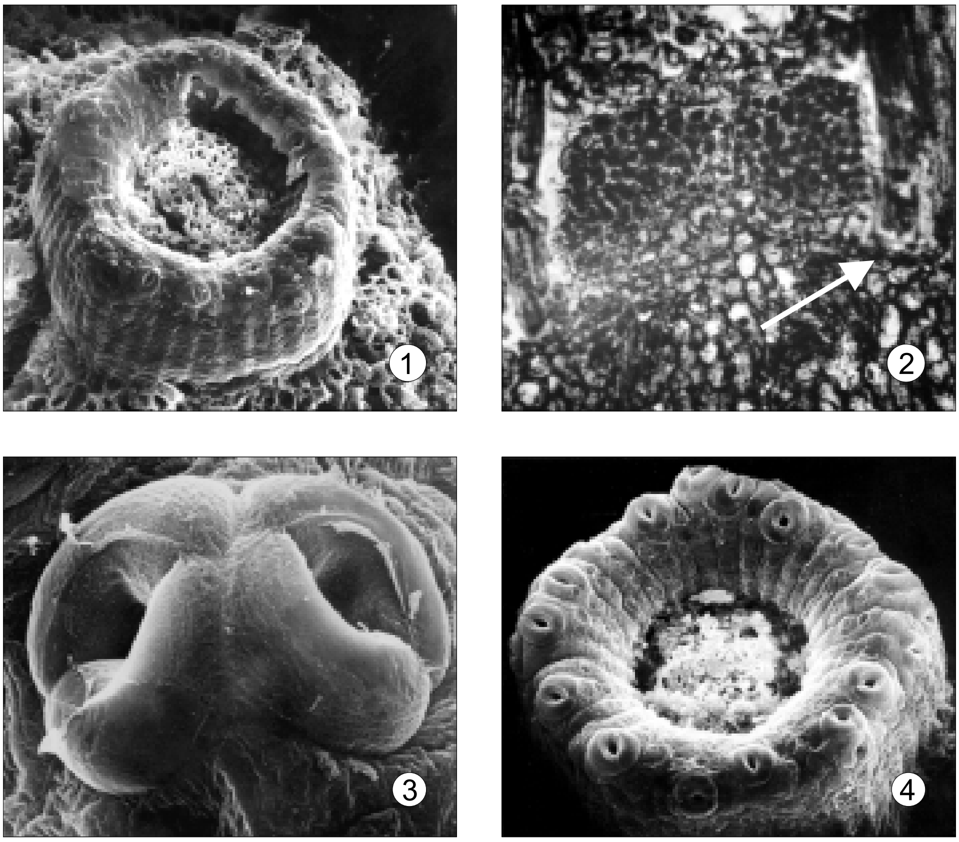
Morphology

For light microscopic tissue examination the inflorescences were fixed in 96% alcohol/glycerine/distilled water (1:1:1), embedded in paraffin, sectioned with a microtome, and stained with toluidine blue.

For scanning electron microscopy, fresh material and samples were fixed with glutaraldehyde and postfixed in osmium tetroxide, both buffered with phosphate buffer. Fixed material was dehydrated, critical point-dried, and coated with a layer of gold. The sections were examined in a scanning electron microscope at 10 kV.

Nectar collection and nectar sugar analysis

The sampling of nectar was made in July and August 1997. Nectar samples were collected in every hour, repeatedly from the same flowers, from previously isolated inflorescence with tulle. The nectar samples were taken from randomly chosen flowers by Whatman no. 1 paper wicks. The temperature and relative humidity were measured over 12 hours. The nectar analysis was carried out by thin layer chromatography (TLC), where the application we used 1 μl Minicaps. Three kinds of sugars were analysed, sucrose, fructose and glucose (others were not present in the nectar samples). We have used standards. The obtained nectar was unfastened from Whatman paper. The mobile phase was ethyl acetate : ethanol : 60% acetic acid-water : saturated with boric acid (5:2:1:1), the post-derivatization took place with thymol sulphuric reagent (Grösz and Braunsteiner 1989). After the chemical reaction had been taken place the purple patches were visible on the TLC plates. Area size of patches was measured with densitometer. The different sugar concentration was counted by area size of patches. Baker-scale based on the proportion of three sugars was used for the characterisation of nectar (Baker and Baker 1975, 1983).



Figs 1–4. 1: *Inula ensifolia*: 1 = Surface view of the nectary with stomata (SEM, ×300); 2 = Longitudinal section of the nectary (LM, ×60) (arrow = ripe cells). 3–4: *Inula salicina*: 3 = Twin-stomata on top of nectary (SEM, ×3,000); 4 = Surface view of nectary (SEM, ×500)

RESULTS

Morphology of nectaries

Their floral nectary functions as an independent compact unit. They are around the basal part of the style, like a ring. Their form is truncated. According to Swales (1979): "The nectaries of the Asteraceae occur as small swellings at the base of the style which fit neatly within the narrow tube of the corolla." The nectaries of the examined species are covered tightly by uniform epidermis cells, which are rectangle-shaped in medial-longitudal engrave and they are in one cell layer and they are covered by the thick cuticle (Figs 5–7). Fahn (1979) studied the morphology and ultrastructure of nectaries and he found: "If the cuticle is very thin from inception, …, the nectar permeates through the cuticle. If the cuticle is thick, it may possess pores; …" Nectar is exuded to the surface via modified stomata (like pores on the nectaries) of gap (Figs 1–8). The radial-anticlinal cell-wall of the epidermis cells are undulatory a bit and they are shown as a vertical columned arrangement from outside (Figs 1, 4). The subepidermal cells are situated irregularly. The glandular tissue is composed of smaller and isodiametric cells with thin walls, relatively large and strongly painted nuclei. No intercellulars among the cells . There is a loose parenchyma tissue toward the basal of the nectary.

According to Pesti (1980) the nectaries of Asteraceae are usually dominated of width, however parameters of nectaries cannot simplify according to examinations. Therefore I constructed categories in order to more exact form and size-determination:

I. the quotient of average breadth (l′) and average height (h′) of the nectaries (= form-determination):

a. isodiametric ($h'/l'=0.9-1.1$): breadth and height of the gland are similar.

b. anisodiametric

i. flattened: $h'/l' < 0.9$

ii. oblong: $h'/l' > 1.1$

According to these categories:

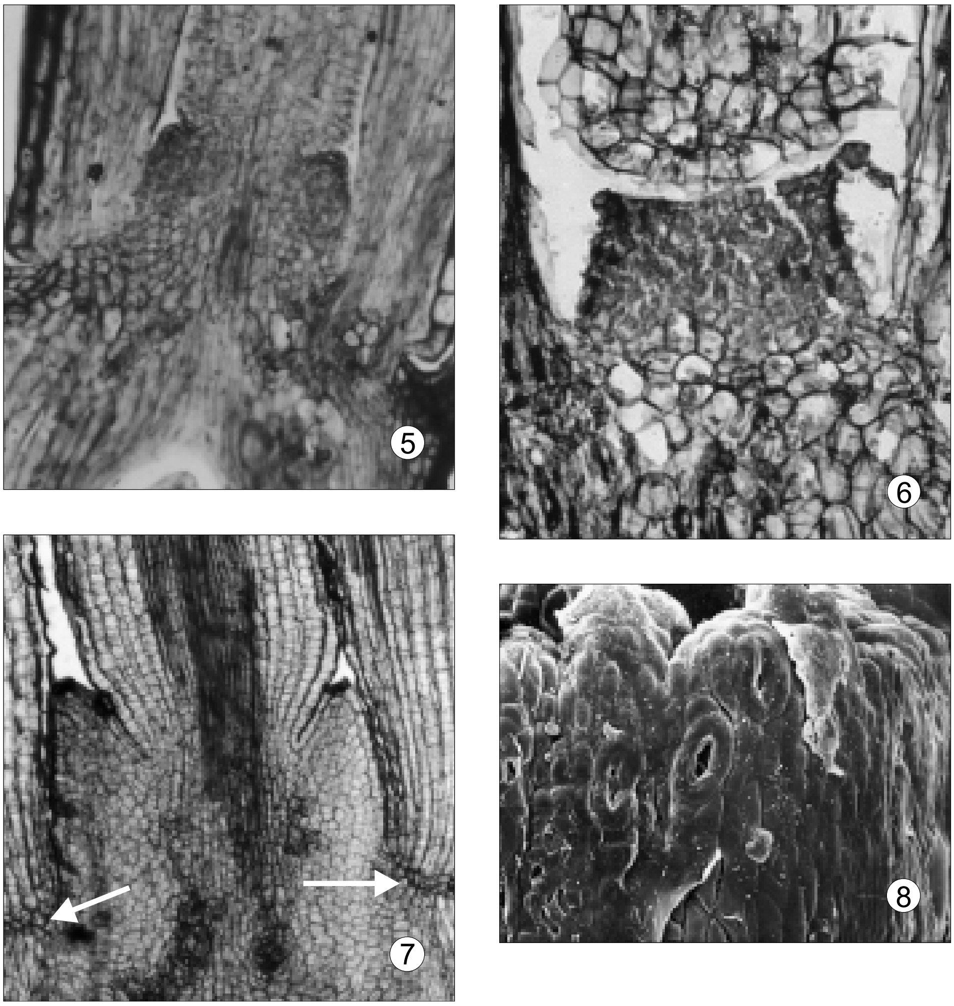
The nectary-breadth is dominated, anisodiametric and flattened: *I. ensifolia* (Figs 1–2), *I. salicina* (Figs 3–5), *I. spiraeifolia* (Fig. 6).

The nectary-height is dominated, anisodiametric and oblong: *C. micranthos* (Fig. 7), *C. scabiosa* (Fig. 8).

II. "Area-multiple", which is formed about average-height of nectary and average-width of nectary in order to complete description (= size-determination).

They have large nectaries (h′· l′ > 100,000 μm²): *C. micranthos* (Fig. 7), *C. scabiosa* (Fig. 8). Their glandular tissue can be found until ovary.

They have small nectaries, the gland tissue is ring-like and it is separeted from neighbouring parts (h′· l′< 100,000 μm²): *I. ensifolia* (Figs 1–2), *I. salicina* (Figs 4–5), *I. spiraeifolia* (Fig. 6).



Figs 5–8. 5 = *Inula salicina*. Longitudinal section of nectary (LM, ×60). 6 = *Inula spiraeifolia*. Longitudinal section of the nectary (LM, ×60). 7 = *Centaurea micranthos*. Longitudinal section of the nectary (LM, ×60) (arrow = ripe cells). 8 = *Centaurea scabiosa*. Lateral view of the upper part of the nectary with stomata (SEM, ×600)

The contracted description is following (form and size determination):
I. Isodiametric, small = IS
Isodiametric, large = IL
II. Anisodiametric
– flattened, small = AFS (*I. ensifolia*, *I. salicina*, *I. spiraeifolia*)
– flattened, large = AFL *(C. micranthos*)
– oblong, small = AOS
– oblong, large = AOL (*C. scabiosa*)

There is a cell-row (so-called "ripe cells") directly under the glandular tissue at the species with small nectaries (Fig. 2), which is found in the nectary-parenchyma at the species with large nectaries (Fig. 7). Along the ripe cells a tube is separated which includes the stamens, style and nectary. We can find the ripe cells only in this phase when the inflorescence is ripening.

The sugar content of nectar in relation to size of nectaries and plant size

The composition of the nectar sugar is examined. Three sugars – the monosaccharides fructose, glucose, the disaccharide sucrose (glucose-fructose) – were encountered in appreciable amounts. Two *Centaurea* species gave very few quantifiable fructose and glucose of appreciable amounts. Among three sugars relative to the ubiquitous sugar sucrose, and the monosaccharides fructose and glucose were not found at every day in the samples, especially at *Centaurea.* The highest sugar content of nectar is produced at the date of the peak flower in the early July in *Inula* species and *C. micranthos* and the nectar production decreased at the beginning of the blooming period and at the end, but it does not leave off. *C. scabiosa* has also been high sugar content at the date of peak flower but it was not continuously on every day.

C. micranthos gives order with the highest content of fructose (31.72%). The highest value was sucrose content/total sugar content in the nectar of *C. scabiosa* (70.09%). The glucose content takes part in sugar content is the biggest in the nectar of *I. spiraeifolia* (40.67%) was the highest, the nectar of *I. spiraeifolia* is hexose-rich (glucose-dominant). The nectar of *I. ensifolia* and *C. micranthos* are sucrose-rich, the nectar of *I. salicina* and *C. scabiosa* are sucrose-dominant according to Baker-scale (S/F+G) (Baker and Baker 1975, 1983, 1990).

The difference in nectar sugar content between two genus was as large as the difference between nectaries size and plants size (Tables 1–2). I found marginally significant positive correlation between the sugar content and plant size ($r_S = 0.6242$; $p = 0.0537$). The nectary size was correlated with the sugar content ($r_S = 0.7547$; $p = 0.0116$).

Table 1
Nectary size of examined species

	Nectary height (h) [μm]	h′ [mm]	Nectary width (l) [mm]	l′ [mm]	h′/l′	h′l′ [mm^2]
Inula ensifolia	110–170	140	230–310	270	0.52	37.800
Inula salicina	100–120	110	230–275	250	0.44	27.500
Inula spiraeifolia	70–80	75	140–180	160	0.46	12.000
Centaurea micranthos	330–460	395	250–380	315	1.25	124.425
Centaurea scabiosa	450–560	505	160–400	280	1.8	141.400

DISCUSSION

According to Pesti (1980) usually width of nectary dominated to Liguliflorae but Tubuliflorae subfamily showed oblong form e.g. *Centaurea* species. Poljakov (1967) (Poljakov's diagram) and later Robinson and Brettell (1973) recognised two major (subfamilies) groups in Asteraceae. This family division into two subfamilies neither represents a basal dichotomy nor a sister group relationship within the Asteraceae. So, the form of nectary has not been indicated as differences between the two subfamilies. The two *Centaurea* species showed different forms (*C. micranthos*: flattened, *C. scabiosa*: oblong).

It is worth noting explicitly that nectar concentration and sugar content values appear to be "conservative characters" at least to the genus level, and perhaps in some cases to higher taxonomic levels, as proposed by Percival (1961). Both the quantity and quality of floral nectar vary among species, and this variation is influenced by environmental factors.

According to Baker and Baker (1990) the nectars of Asteraceae are dominated by hexose sugars. Sugar content in four species has been shown sucrose dominant and I found one species which is glucose dominant.

Although the number of examined species are a few, the correlation may reflect greater allocation to sugar content by larger plants and larger nectaries.

Table 2
Average of the daily total sugar content of examined *Inula* and *Centaurea* species, and the Baker-scale

	Inula			*Centaurea*	
	ensifolia	*salicina*	*spiraeifolia*	*micranthos*	*scabiosa*
Sugar content (mg/ml)	18.5008	22.1348	9.2103	58.6527	34.2068
Baker-scale (S/(F+G))	0.9915	1.41	0.4253	0.9075	2.3443

*

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