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NECTAR AND NECTARY STUDIES ON SEVEN *EUPHORBIA* SPECIES

N. PAPP

Department of Botany, University of Pécs
H-7624 Pécs, Ifjúság útja 6, Hungary; E-mail: nora4595@freemail.hu

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The nectary morphology of six Hungarian and one grown Mediterranean leafy spurge (*Euphorbia*) species was studied and compared. Glands with various horns were found in *E. amygdaloides* L., *E. cyparissias* L., *E. esula* L., *E. myrsinites* L. and *E. virgata* W. et K. Nectary without horns is characteristic to *E. palustris* L. and *E. polychroma* Kern. Cuticle pattern of the glands was different and characteristic to the species. *E. amygdaloides* has no cuticle wrinkles. Special letter shaped wrinkles were found in the species, for example "H" shaped in *E. cyparissias*, *E. esula*, *E. palustris* and *E. virgata*. Wrinkles in *E. myrsinites* have several branches; they could be "Z", "W", "Y" and "E" shaped. The nectar of each studied plants contained fructose, glucose and sucrose. An unknown sugar was found in *E. cyparissias* only.

Key words: *Euphorbia*, nectary, cuticle pattern, nectar component

INTRODUCTION

The family Euphorbiaceae has various types of inflorescence and pollination in the five subfamilies. Nectaries are not characteristic to each subfamily because of different pollination types. Double perianths occur in the ancient subfamilies, becoming reduced to nude inflorescences in the younger subfamilies (Borhidi 1993). Double perianth is characteristic to the genera *Phyllanthus* (subfam. Phyllanthoideae) and *Croton* (subfam. Crotonoideae). Glands found in the tropical genus *Jatropha* originate from the bracts.

Nectaries occur mainly in the subfamily Euphorbioideae. *Pedilanthus tithymaloides* has a spur-like nectary, which is half mm high and has 3 parts: a head, a neck and a style. External cells of the head secrete the nectar, which contains sugar, starch and tannin. Glands of *Synadenium* sp. grow together as a ring. Nectar of *Euphorbia pulcherrima* contains 60% of sugar. Besides secretion and storage, carbohydrate transformation also takes place in the nectary (Danert *et al.* 1976).

A special inflorescence, the so-called cyathium was described in the genus *Euphorbia*. This reduced monochlamydeous inflorescence has bracts while the perianth misses. Bracts often become red to enhance insect attraction. Nectaries are nuptial and extrafloral. They develop unprotected with their

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large free exposed surface. Generally there are four nectaries alternately between the bracts. The fifth nectary often misses where the pistillate flower bends down. These nectaries start to secrete after the maturity of the pistillate flower (Arumugasamy *et al.* 1990). Nectary of *Euphorbia seguieriana* Necker is accessible for insects. This leafy spurge was described as a good honey producer of the genus. This production concurs with that of *Robinia* sp., but it is rich only every 4–5 years (Örösi 1968). Most of the glands in the genus are half moon shaped with or without horns. The studied seven species have different life strategy at variant habitats. *Euphorbia amygdaloides* L., *E. cyparissias* L., *E. esula* L., *E. palustris* L., *E. polychroma* Kern. and *E. virgata* W. et K. are common in Hungary; they have a lot of insect visitors (Meeuse *et al.* 1989). Spiders visit *Euphorbia palustris* L. and *E. esula* L. (Vroege *et al.* 1987). A Mediterranean species, *Euphorbia myrsinites* L. was also studied for its nectary characteristics.

The nectaries, differing in size and shape, have variant cuticle pattern with different number of stomata at the species. The nectar gets out from the glandular tissue to the surface through nectary stomata. On the surface several cuticle wrinkles enhance spreading of the nectar, acting as capillaries (Orosz-Kovács 1991).

The aim of the study was to describe some morphological parameters and nectary surface pattern and to determine the sugar content of the nectar.

MATERIALS AND METHODS

Studied plants

The seven *Euphorbia* species were collected in May 2002 in the environs of the Mecsek (Árpád-tető, Pellérd), a mountain in south Hungary. Different habitats of the collection were the following:

Euphorbia amygdaloides L. Habitat: a forest in Árpád-tető. – This overwintering species lives in shadow in forest (Simon 2000).

Euphorbia cyparissias L. Habitat: an open glade in the Mecsek Mts. – This perennial plant prefers xerophilous habitats. Besides propagating by seeds, it reproduces vegetatively by rhizomes (Simon 2000). Several pollinators participate in the fertilisation.

Euphorbia esula L. Habitat: a forest edge in Árpád-tető. – It is common along roads. Insect pollination is important at this species (Simon 2000).

Euphorbia myrsinites L. Habitat: the Botanical Garden of the University of Pécs. – It is a Mediterranean species with thick succulent leaves and stem (Săvulescu 1953). It is frequently planted as an ornamental plant in gardens of Hungary.

Euphorbia palustris L. Habitat: a humid field in Pellérd. – Some of these tall plants form great bushes on humid fields and swamps (Simon 2000). Some insects and spiders play a role in pollination (Vroege *et al.* 1987).

Euphorbia polychroma Kern. Habitat: a forest edge in Árpád-tető. – It prefers forest and road edges (Simon 2000). Leaves as well as bracts, become red in autumn.

Euphorbia virgata W. et K. Habitat: a road edge in Árpád-tető. – It is very similarly to *E. esula* L., but it is taller and has more branches (Simon 2000).

Treatment of the samples

A mixture of 50 ml distilled water and 6 g p-formaldehyde was warmed to 60 °C. For purification 2 drops NaOH, then 5 ml 0.4 M kakodylate buffer was added to the solution. The pH was set to 7. Fresh cyathia (4–5 cyathia/individual/species) were soaked and kept in this mixture for 22 hours. Washing followed in kakodylate buffer three times for 60–60 minutes, then in distilled water for 60 minutes. 800 µl OsO_4 was added to each sample; they were kept in this for 24 hours and then in distilled water for 60 minutes. Dehydration followed in alcohol series (25%, 50%, 70%, 90%, 96%), samples being kept in each for 12 hours; in the end in absolute alcohol four times for 60–60 minutes. They were washed in amyl acetate three times for 2 hours. Dehydration was completed in a critical point dryer. Sample surfaces were sputter coated with a thin layer of gold. Nectary photos were taken with scanning electron microscope (SEM) in the laboratory of the Faculty of Medicine in June 2002.

Morphological measurements on SEM micrographs were carried out with the software Image Tool 2.1. Each characteristic was measured on 10 samples. Means and SD values were calculated from 10 measurements. The measured characteristics were the following (Fig. 1, Table 1):

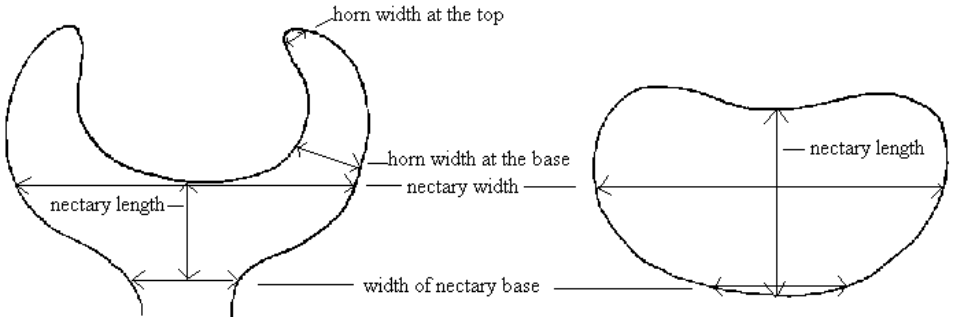

Fig. 1. Measured parameters of the glands (drawing by Nóra Papp)

Table 1
Morphological characteristics and SD values of the nectaries of the studied *Euphorbia* species

	E. amygdaloides L.	*E. cyparissias* L.	*E. esula* L.	*E. myrsinites* L.	*E. palustris* L.	*E. polychroma* Kern.	*E. virgata* W. et K.
Nectary area (103 µm²)	962.49± 9.70	719.61±59.07	240.57±21.36	1566.54±276.84	608.19±16.62	758.16± 162.42	1790.17±285.41
Nectary width (µm)	1389.70± 142.00	1006.00±136.90	1246.80±115.00	1945.40±61.50	1084.30±17.13	941.59± 34.60	1648.97±284.00
Nectary length (µm)	482.38± 16.50	725.87±55.90	703.56±41.30	919.21±19.10	640.13±17.65	1031.42±36.80	8 57.62±46.00
Width of nectary base (µm)	502.61± 102.60	374.48±51.50	540.81±72.40	729.37±87.70	403.59±108.18	419.48±36.70	833.46±53.40
Width of nectary horn at the base (µm)	384.68± 85.92	176.05±23.60	424.09±74.60	96.29±19.10	–	–	538.24±62.30
Width of nectary horn at the top (µm)	147.53± 13.90	51.14±13.40	102.22±18.50	375.24±158.22	–	–	134.10±21.00
Length of nectary horn (µm)	653.51± 91.00	118.03±23.47	440.24±85.50	756.20±59.70	–	–	890.02±73.40
Mean of stoma number / 10000 µm²	1	3	1	3	3	4	1
Number of cuticle wrinkles / 10000 µm²	–	188±25	170±13	138±21	187±31	273±45	342±38
Number of cuticle wrinkles / one cell	–	2–7	4–9	2–5	4–7	2–5	4–7
Distance of cuticle wrinkles (µm)	–	1.43±0.20	1.60±0.31	1.66±0.38	2.60±0.24	1.64±0.18	1.91±0.30
Number of cells around one stoma	5	7	5	8–9	5–6	7	8

– width and length of the nectaries,
– width of nectary base,
– width of horns at the base and at the top,
– horn length,
– nectary area,
– number of stomata and cuticle wrinkles per cells and per unit area (10,000 μm^2),
– distance of cuticle wrinkles,
– number of cells around one stoma.

Wrinkles were characterised according to their shape, branches and direction.

Qualitative analysis of the nectars

For nectar analysis 20 fresh flower heads per species were collected in the morning hours in May. Thin layer chromatography was used to study the sugar contents of the nectars. The flower heads were washed with 5 ml methanol (Damon *et al.* 1999). These extracts were applied to silica gel plate with microcapillaries (10 µl each). The standards were the following: fructose, glucose, sucrose, raffinose, arabinose, galactose, xylose and maltose (3 µl each). The mobile phase was a solvent mixture: ethyl-acetate : ethanol : 60% acetic acid : water coldly saturated with boric acid (50:20:10:10). First development was carried out in 30 ml of this solution, the second in 60 ml the next day. After development the plate was dried at 105 °C for 5 minutes. It was developed with Thymol-reagent (0.5 g thymol dissolved in 95 ml ethanol, then 5 ml *cca* H_2SO_4 was added to them). Following another drying at 105 °C for 5 minutes, the plate was visualised under UV light (366 nm) (Papp 2003).

RESULTS

Morphological characteristics

The yellow nectary surface glistens from the nectar at the beginning of flowering in each species. Afterwards it becomes dry and unpolished. Colour can change from yellow to orange or brown at the end of nectar production.

Gland of *E. amygdaloides* has long and sharpened horns (Fig. 2a), between which the glandular tissue is located. Due to the dominance of vegetative reproduction the surface of its nectary is small. The shortest nectary appendage was found in *E. cyparissias* (Fig. 2b), the longest in *E. virgata* (Fig. 2g). Horns in *E. cyparissias* may differ in size: in some cases they are as long as the nectary, in

other cases smaller than the total width of nectary. The horn top in *E. myrsinites* was rounded, similarly to *E. virgata* (Fig. 2d, g). The oval glands of *E. palustris* and round nectary of *E. polychroma* have no horns (Fig. 2e, f).

Euphorbia myrsinites and *E. virgata* had the widest nectaries, whereas *E. polychroma* the smallest one (Table 1). The ratio of the width and length of the glands was generally 1:2 or 1:3, in the round gland of *E. polychroma* it was 1:1. Stomata were visible only on the nectary surface of *E. amygdaloides*, because the cuticle has no wrinkles here. Several cuticle wrinkles are arranged around the stomata of the other species, *E. virgata* having the most of them. The stomata were surrounded by the largest number of cells in *E. myrsinites* and *E. virgata*, which have the biggest nectary area. Distance of the wrinkles was the biggest in *E. palustris*.

Cuticle pattern

Primary and secondary types of the wrinkles were distinguished. The primary ones, called striae, are located directly on the nectary cell surface. The

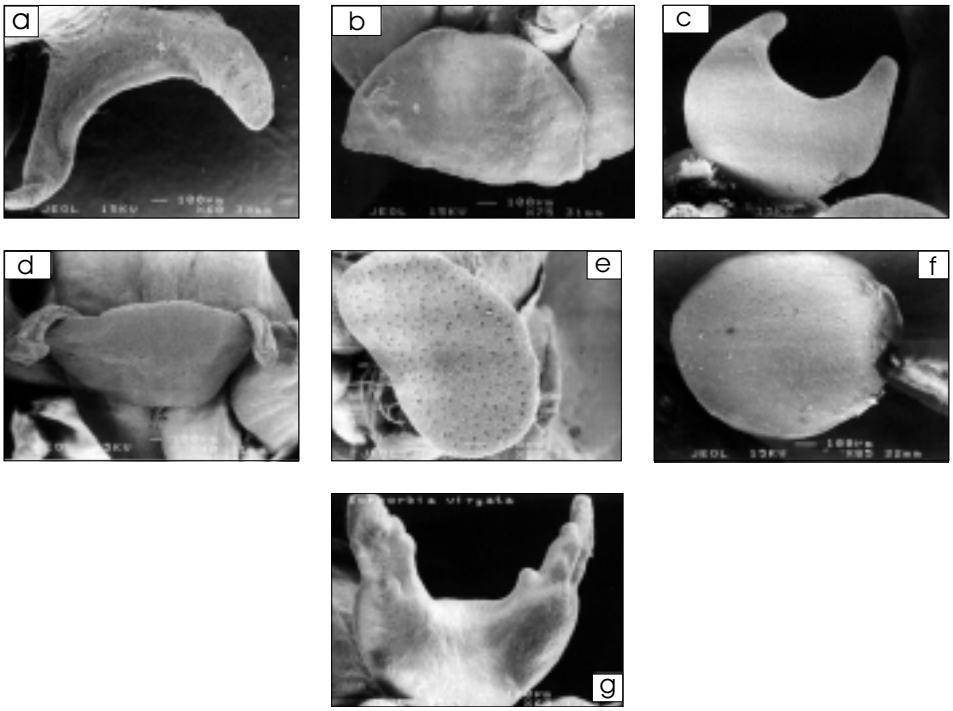

Fig. 2. Whole nectaries of the leafy spurges (a = *E. amygdaloides*, b = *E. cyparissias*, c = *E. esula*, d = *E. myrsinites*, e = *E. palustris*, f = *E. polychroma*, g = *E. virgata*)

secondary ones, called crests, spread on the primary striae. Crest belongs to the reticulate cuticle type (Metcalfe and Chalk 1979).

Wrinkles were arranged radially around the stoma in each species. Glands of *E. amygdaloides* have no cuticle wrinkles. Mesomorphic stomata are surrounded by 5 cells (Fig. 3a).

Slightly sunken xeromorphic stomata of the nectary in *E. cyparissias* and *E. esula* were limited by 5–6 cells (Fig. 3b, c). Most of the wrinkles are primary striae with some crests (Fig. 4a, b). Wrinkles were slightly wavy and arched without branches. A special "H" shaped wrinkle is characteristic to the both species. Wrinkle number can change between 2–5 per cells in *E. cyparissias* and 3–5 in *E. esula*.

Parallel wrinkles are characteristic in the interstomatal area, whereas radial ones are typical around the stoma in *E. myrsinites* (Fig. 3d). The exclusively primary striae have different shapes: they can branch abundantly and close with themselves (Fig. 4c). They can be "Z", "W", "Y" and "E" shaped, too.

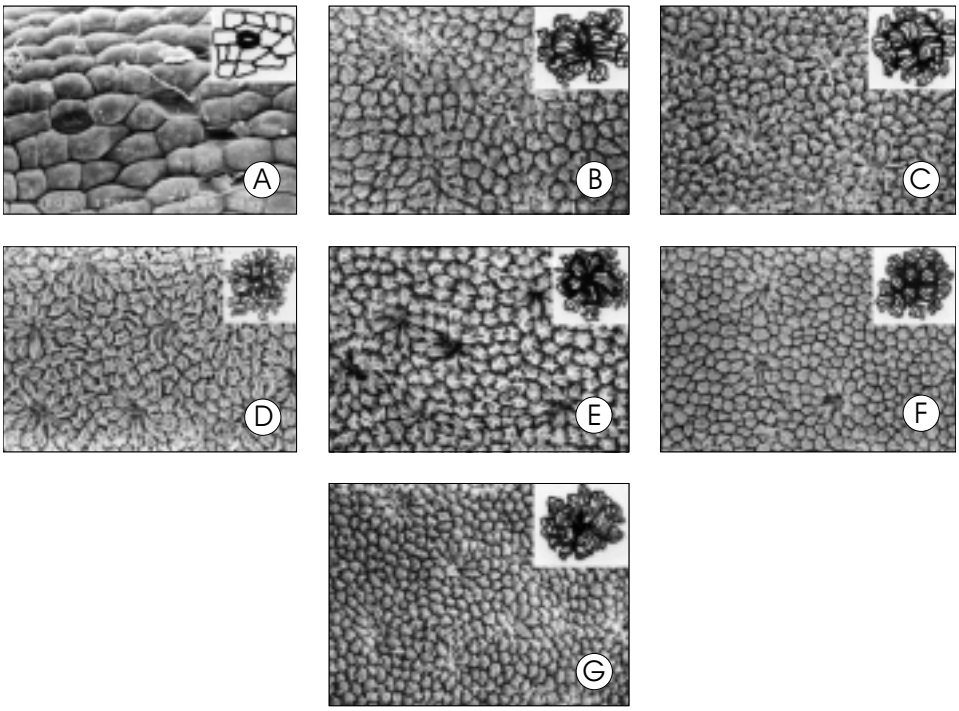

Fig. 3. Nectary surface and cuticle wrinkles around one stoma (drawing by Nóra Papp) (A = *E. amygdaloides*, B = *E. cyparissias*, C = *E. esula*, D = *E. myrsinites*, E = *E. palustris*, F = *E. polychroma*, G = *E. virgata*)

Only one or two wavy wrinkles were found per cell. Striae are wide and separate sharply from each other.

Primary striae and secondary crests were also found on the gland cuticle of *E. palustris* (Fig. 4d). They are wavy, arched with branches, frequently "F" and "H" shaped. Big distance was measured between the wrinkles (Table 1). Mesomorphic stomata are surrounded by 5–6 cells (Fig. 3e). No stoma can be observed along the edge of the gland (Fig. 2e).

The primary cuticle striae in *E. polychroma* are compact and short without branches and secondary crests (Fig. 4e). Most often they are straight, some-

Fig. 4. Cuticle wrinkles on the nectary cells (drawing by Nóra Papp) (a = *E. cyparissias*, b = *E. esula*, c = *E. myrsinites*, d = *E. palustris*, e = *E. polychroma*, f = *E. virgata*)

where slightly arched. The striae number varied between 1–5 per cell. 6–8 cells limit its mesomorphic stoma (Fig. 3f).

Cuticle wrinkles are strongly wavy in *E. virgata* (Fig. 4f). Primary striae have secondary crests too, 2–5 per cell. Distance between the wrinkles was big, similarly to the cuticle of *E. palustris* (Table 1). Xeromorphic stomata are limited by 5–6 cells (Fig. 3g).

Sugar contents of the nectars

The nectar components of each studied plant were the following: fructose (Rf value = 0.58), sucrose (Rf = 0.63) and glucose (Rf = 0.66) (Fig. 5). Other tests were not present in the secretory product. Spot intensity of the plate indicates that fructose was found in the highest and sucrose in the lowest quantity among sugars in the nectar. *E. amygdaloides* had the most sucrose, the least was found in *E. myrsinites* and *E. cyparissias*. The most glucose occurred in *E. myrsinites*. *E. cyparissias* had an unknown sugar component (Rf = 0.41) which was not found in the others.

DISCUSSION

In the absence of previous studies it was necessary to investigate the nectary surface, morphology and nectar composition of *Euphorbia* species. It was stated that nectary surface patterns are characteristic to the studied species. The main sugar components (fructose, glucose and sucrose) were deter-

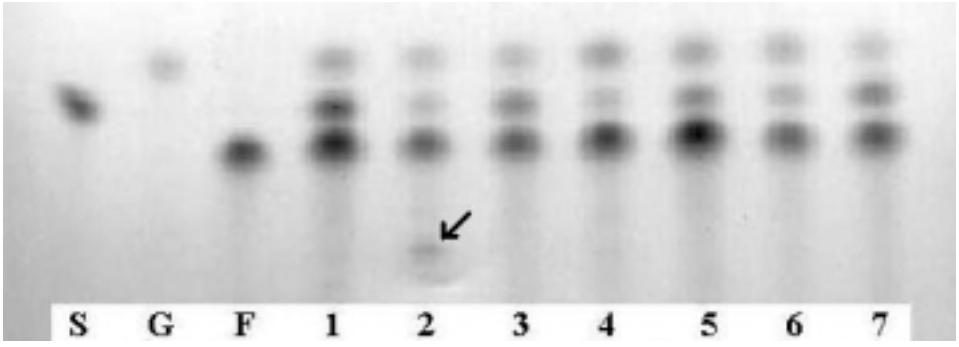

Fig. 5. Standards (**S**: sucrose, **G**: glucose, **F**: fructose) and sugar components of the nectar (1 = *E. amygdaloides*, 2 = *E. cyparissias*, 3 = *E. esula*, 4 = *E. myrsinites*, 5 = *E. palustris*, 6 = *E. polychroma*, 7 = *E. virgata*). The arrow indicates an unknow compound in the nectar of *E. cyparissias*

mined, but further studies are needed for identifying an unknown compound in *E. cyparissias*.

*

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