FLOWERING BIOLOGY AND NECTARY STRUCTURE OF *Melissa officinalis* L.

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**Abstract**

The present study on lemon balm (*Melissa officinalis* L.) covered flowering biology, monitoring of pollinating insects and floral nectary structure. The micromorphology of epidermal cells of the nectary was investigated using scanning electron microscopy. The nectariferous tissues were observed using light microscopy based on semi-thin sections.

Lemon balm flowered from the second decade of June until September. Buds opened from early morning hours until noon. Flowers lived for 24 hours, on the average. Their primary pollinator was the honey bee. The beginning of nectar secretion was found to be at the bud swell stage. The automorphic nectary forms a disc with four protrusions at the base of the nectary. Three smaller ones and one larger than the other ones were distinguished among them. No stomata were found on the lower protuberances, whereas on the highest part anomocytic stomata were present, the number of which was 15. The stomata exhibited different development stages and they were situated above other epidermal cells. In their outline, they were ellipsoidally shaped (18 x 23 μm) and they had average-sized cuticular ledges. They produced a smooth cuticle and wax granules. In cross section, the nectary tissues were composed of a single-layered epidermis and 9 – 11 layers of the nectary parenchyma. Their thickness was 198 μm. In longitudinal section, the height of the nectary was within a range of 354 – 404 μm. The epidermal cells produced thin outer cell walls. Some of them were completely filled with strongly stained cytoplasm, whereas others showed a high degree of vacuolisation. But the nectary parenchyma cells were marked by poorly stained cytoplasm, a large nucleus and vacuolisation of varying degree.

**Key words:** *Melissa officinalis*, blooming, visiting insects, nectary, micromorphology, stomata, anatomy

**INTRODUCTION**

Lemon balm (*Melissa officinalis*) is native to southern Europe, Asia Minor and the Caucasus (*Szwedkowscy*, 2003). In Poland in the 16th century, lemon balm used to be planted in monastery gardens (Góra and Lis, 2007). *Melissa officinalis* releases lemon-scented essential oil which contains active substances, among others: citronellal, citronellol, geranial, neral and linalol (*Vaverkova et al.* 2002; Góra and Lis, 2007; Adenie *et al.* 2008; Cosge *et al.* 2009). Thanks to these compounds, lemon balm is classified as an important medicinal, spice and cosmetic plant; it also exhibits sedative, antiseptic and antihistaminic activity (Nemeth and Szekely, 2000; Muntean and Marian, 2005; Bahtiyarca and Cosge, 2006; Adenie *et al.* 2008). Due to aromatic values, valuable pharmacological components as well as the ornamental and apicultural value of its flowers, lemon balm is often grown in vegetable and herb gardens (*Squire*, 2007; Sikora, 2008). In literature, honey yield of lemon balm is estimated to be 32 – 150 kg/ha (Demianowicz, 1953; Bahtiyarca and Cosge, 2006; Muntean and Marian, 2005; Kotlinowski, 2006).

Nectar secretion onto the surface of the nectary occurs through stomata in flowers of Lamiaceae plants. The nectaries in Lamiaceae form, at the base of the nectary, a disc which, depending on the taxon, differs in its size, symmetry, shape and number of protrusions (*Dafni et al.* 1988; Weryszko-Chmielewska, 2000; Weryszko-Chmielewska and Chwil, 2002; Chwil, 2007). Based on these traits, Weryszko-Chmielewska (2000) distinguished three groups of floral nectaries in plants of the family Lamiaceae. However, Petanidou *et al.* (2000) report that the nectary structure in this family is determined to a great degree by phylogenetic and climatic conditions.

The aim of the present study was to determine flowering biology, groups of pollinating insects and nectary structure in the flowers of *Melissa officinalis*.
MATERIALS AND METHODS

The study of lemon balm (Melissa officinalis L.) flowers was carried out in the years 2006 – 2007 in the Botanical Garden of the Maria Curie-Skłodowska University in Lublin. Flower lifetime and the daily rate of flower opening were estimated as well as flower visitation by insects was monitored.

For microscopic analysis of the nectaries, portions of flowers at full bloom stage were sampled. The structure of the nectaries was investigated using stereoscopic, light and scanning electron microscopy. The micromorphology of epidermal cells of the nectary was analysed under a BS-300 Tesla scanning electron microscope. The plant material for SEM analysis was fixed in 4% glutaraldehyde and 0.1 M phosphate buffer with a pH of 7.2. After being treated with 1% osmium tetroxide and dehydrated in acetone series, the samples were critical-point dried and then coated with gold using a sputter coater.

The nectariferous tissues were observed under a light microscope based on semi-thin sections. After postfixing, the plant material was dehydrated in alcohol series and embedded in Spurr’s low viscosity embedding resin. 0.5 – 0.7 μm sections were cut on a Reichert Ultracut S ultramicrotome. Then, they were stained with 1% toluidine blue with 1% azur II in a 1% aqueous solution of sodium tetraborate. Observations and photographs were made with a Nikon YS100 microscope.

RESULTS

Flowering and flower visitation by insects

In the climatic conditions of Lublin, lemon balm flowered from the second decade of June until September. In the upper part of its stems, it produced white flowers, clustered in one-sided pseudo-whorls with 3 – 6 flowers in each. Numerous hairs were growing on the surface of the perianth elements (Figs 3A, B).

Buds opened from early morning hours until noon. The process of flower opening from the bursting bud until the generative elements reached maturity lasted about 30 minutes. The studied flowers lived for 24 hours, on the average. Following senescence, the corolla abscised, whereas the sepals remained on the plants and performed a protective function for the fruits.

The greatest intensity of blooming was observed in morning hours. The number of open flowers until 900 accounted for 66% of all flowers which opened during the day. But between 900 and 1100, one half fewer flowers bloomed. In afternoon hours, from 1300 to 1500, new single flowers opened only sporadically (Fig. 1).

The lemon balm flowers were visited by several groups of insects, among which the honey bee was the primary pollinator. The largest number of pollinating insects was observed between 900 and 1200. The percentage proportions of honey bees, solitary bees and bumblebees in the pollination of lemon balm flowers were, respectively: 45%, 18% and 13%. Other pollinating insects (dipterans and butterflies) accounted for 24% of the pollinators observed (Fig. 2).
Nectary micromorphology

The beginning of nectar secretion in the lemon balm flowers was found to be at the bud swell stage. Nectar is produced by the automorphic nectary located at the base of the four-parted ovary (Figs 3C – F). The nectariferous tissue of lemon balm forms an asymmetric disc with four protrusions which enter between the particular parts of the four-parted ovary. Among these protrusions, three smaller ones and one larger than the other ones were distinguished. No stomata were found on the lower protuberances (Figs 3D – F). In these regions, the epidermal cells produced a smooth cuticle with slight folding (Figs 3E, G). The height of particular parts of the nectary ranged between 354 and 404 μm. The convex part with stomata was elevated above the other portions of the gland up to a height of approx. 70 μm. The diameter of the nectary in its widest part was within a range of 657 – 687 μm, reaching an average value of 677 μm.

In the highest part of the nectary, clusters of anomocytic stomata were found, which were responsible for nectar secretion (Fig. 3F, H). The average number of stomata on a single protrusion was 15. The stomatal cells were arranged irregularly and they demonstrated different development stages. There occurred both closed stomata, with the swollen cuticle visible over the pore, and open stomata with different pore apertures (Figs 3H, 4A – I).

The stomata, surrounded by 5 – 7 cells, were situated above other epidermal cells. In their outline, they were ellipsoidally shaped (18 x 23 μm) and they had average-sized cuticular ledges. They produced a smooth cuticle, which was slightly folded on other epidermal cells of the nectary (Figs 4A – I). On the nectary’s protrusion with the stomatal region, there occurred wax granules, at places forming larger irregular clusters (Figs 4A, B, D – F).

Nectary anatomy

In cross section, the nectary tissues were composed of a single-layered epidermis and 9 – 11 layers of the nectary parenchyma. These cells formed a compact arrangement. Their thickness was within a range of 182 – 236 μm, reaching an average value of 198 μm. The epidermal cells of the nectary were isodiametrically shaped. They produced thin outer cell walls. Among them, cells filled with strongly stained cytoplasm were visible, or highly vacuolised brighter ones (Figs 5A, C).

The walls of the nectary parenchyma cells were thin. The cells of this layer were marked by poorly stained granular cytoplasm, a large nucleus and vacuolisation of varying degree (Figs 5A – C). The parenchyma cells of the receptacle formed a looser arrangement; they were larger than the nectary parenchyma cells. Around a large vacuole, they produced a thin parietal strand of cytoplasm (Figs 5A, B, D).
Figs 3. Flowering plants (A, B) and portions of the nectary (n) with the ovary (o) of *Melissa officinalis*.  
C – nectar droplet visible on the surface of the nectary (asterisk).  
D – H – nectary’s protrusions without stomata (arrowhead) and with the stomatal region (arrows).  
C, D, F – bars = 200 μm; G – bar = 10 μm; H, E – bars = 50 μm.
Fig 4. Portions of the nectary epidermis with the stomatal region in *Melissa officinalis* flowers.

A – from the side of the ovary (o). Visible wax granules, at places forming larger structures (arrow). Bar = 50 μm.
B – in the apical part.
DISCUSSION

The studied lemon balm flowered for over two months. On the average, flowers lived for 24 hours. They bloomed most intensely in morning hours. Bees and bumblebees were the primary pollinators of the studied lemon balm, described in literature (Bożek, 2000). Literature reports show that flowers of numerous plant species of the family Lamiaceae are pollinated mainly by bees (Dafni, 1991; Petanidou and Vokou, 1993).

This is also confirmed by studies on flowers of several Ocimum species, in which Apis mellifera visits accounted for 50-98% (Malerbo-Souza et al. 2000; Chwil, 2007). In the studies of Dafni et al. (1988), among 9 plant species of the family Lamiaceae, most frequent visits of bees were found in the flowers of Rosmarinus and Coridothymus capitatus. In explaining the pollinator-flower interaction, the authors divided the investigated taxa into 3 groups which differed in the sugar composition of nectar. The flowers of Melissa and Rosmarinus, in whose nectar the amount of hexoses was equal to sucrose, were included in the first group; in the second group hexoses were predominant (Coridothymus, Salvia judaica and Stachys), whereas in the third one the largest amount of sucrose was found (Phlomis, Salvia fruticosa and Satureja).

The nectary in the investigated flowers of lemon balm forms, at the base of the ovary, an asymmetric disc with four protrusions with a height of 354 - 404 μm and a diameter of 677 μm. It differs compared to the nectaries of other species of the family Lamiaceae described in literature. It is higher than the nectary gland of Origanum and Mentha, but lower than that in Nepeta, Hyssopus and Ocimum (Weryszko-Chmielewska, 2000; Chwil, 2007; Mačkanović-Jocić et al. 2007). On the other hand, its diameter was smaller by 50 up to 70% than the diameter of the nectaries of Ocimum (Chwil, 2003, 2007; Mačkanović-Jocić et al. 2007). According to Mačkanović-Jocić et al. (2007), cells of the nectary in basil flowers in-
creased their diameter during ontogeny. Other authors report that the nectary size in the flowers of Lamiaceae is positively correlated with nectar secretion, but not with its quality. Larger diameter nectaries are found in Lamiaceae flowers with a wide corolla and they develop large, open pores, whereas in deep flowers no such correlation has been demonstrated (Dafni et al. 1988; Petanidou et al. 2000).

The stomata in the epidermis of the nectary of lemon balm exhibited different development stages. This suggests the possibility of regulating the pore aperture during nectar secretion. Petanidou et al. (2000) report that the process of pore opening in the nectaries of other plant species of the family Lamiaceae decreases at the time of anthesis, and at the same time it affects the nectar production rate and sugar concentration in nectar.

In the stomatal region of the nectary of the lemon balm flowers observed, 15 stomata were found on the average. It is known from literature data that the number of developed stomata on a protrusion of the nectariferous tissue in flowers of different taxa of the family Lamiaceae is variable. Their lowest number has been found in the epidermis of the nectaries of the genera Nepeta (4 – 6) and Mentha (7 – 8), whereas higher stoma density has been observed in Ocimum (4 – 19), Hyssopus (17 – 18) and Origanum (15 – 18) (Weryszko-Chmielewska, 2000; Chwil, 2007; Maćkkanović-Jocić et al. 2007).

Ellipsoidal (18 x 23 μm) modified stomata in the nectary epidermis of lemon balm belonged to the anomocytic type. Their shape and size as well as their position are similar to those of diacytic stomata in leaf epidermis of this taxon described in an earlier paper (Chwil 2006). The location of stomata above other epidermis of this taxon described in an earlier paper (Chwil 2006) is confirmed by the studies of Dafni et al. (1988) relating to the flowers of Melissa officinalis, Coridothymus capitatus and Salvia thymbra.

The epidermal cells of the nectary of lemon balm produced a smooth or slightly folded cuticle as well as wax granules.

In cross section, the nectary tissues in the investigated Melissa officinalis flowers were composed of a single-layered epidermis and 9 – 11 layers of the nectary parenchyma. Their thickness was 198 μm, on the average. A similar number of cell layers (10) was found in the nectaries of Ocimum (Maćkkanović-Jocić et al. 2007).

**REFERENCES**


Biologia kwitnienia i budowa nektarnika Melissa officinalis L.

Streszczenie

Przeprowadzone badania melisy lekarskiej (Melissa officinalis L.) obejmowały biologię kwitnienia, monitoring owadów zapylających i strukturę nektarnika kwiatowego. Mikromorfologię komórek epidermy nektarnika analizowano w skaningowym mikroskopie elektronowym. Tkanki nektarnikowe obserwowano w mikroskopie świetlnym w oparciu o preparaty półcienkie.