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MORPHOLOGY AND ANATOMY OF FLORAL NECTARY AND COROLLA OUTGROWTHS OF *MYOSOTIS SYLVATICA* HOFFM. (BORAGINACEAE)

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The structure of nectaries in the flowers of *Myosotis sylvat*ica Hoffm. was studied by light microscopy, scanning electron microscopy and transmission electron microscopy. A nectariferous gland of irregular disc shape surrounds the bottom of the four-lobed ovary. From the upper side, both the nectary and the ovary are protected by ligulate folds of a widening corolla tube with epidermis outgrowths. Nectar is secreted by modified stomata situated only on the top and abaxial nectary surface adjoining the corolla tube. Stomata are irregularly distributed, forming aggregates of 2–3 each. On the longitudinal sections of the receptacle, the nectariferous tissue is distinguished by small cells, dense protoplast content, and intercellular spaces of different sizes. Branches of phloem bundles are observed at the base of the nectariferous tissue.

Key words: *Myosotis sylvatica* Hoffm., Boraginaceae, nectaries, corolla outgrowths, morphology, anatomy.

INTRODUCTION

The flowers of *Myosotis* L. are visited by bees, butterflies and flies (Maurizio, 1953; Kugler, 1970; Proctor et al., 1996). Honeybees forage on their nectar and pollen (Maurizio and Louveaux, 1963). Data on the biology of anthesis and pollination of six other species of the genus *Myosotis* native to New Zealand were given by Robertson and Lloyd (1991, 1993) and Robertson and Macnair (1995), who stated that all investigated species are protogynous and that the stigma remains functional throughout the life of the flower. The flowers of this taxon are pollinated by some Diptera.

Nectaries in the flowers of Boraginaceae form a disc-like ring below the ovary (Kugler 1970). According to Fahn (1952) and Weberling (1992), discoid nectaries are classified as receptacular, developed as outgrowths of the receptacle.

This study looked into the morphological and anatomical traits of the nectaries of *Myosotis sylvatica* Hoffm. and the structure of the accompanying outgrowths (folds) of the corolla at anthesis.

MATERIALS AND METHODS

Entire inflorescences of *Myosotis sylvatica* Hoffm. were collected from plants grown in a garden in Lublin, Poland, and examined by bright-field light microscopy (LM), transmission electron microscopy (TEM) and scanning electron microscopy (SEM).

The lower parts of the flowers (with corolla tube and nectary) were fixed at anthesis in 3% glutaraldehyde in 0.1 M phosphate buffer (pH 7.0) for 4 h at room temperature. The material was rinsed in two changes of buffer, post-fixed in 2% osmium tetroxide for 4 h at 4˚C, rinsed again in buffer, and dehydrated in an ethanol series and acetone prior to embedding in Spurr's resin. Semithin sections 1µm thick were cut for light microscopy with a Reichert Ultracut S microtome and stained with 1% methylene blue and 1% azure II. Ultrathin sections for TEM were stained with uranyl acetate followed by lead citrate, and viewed with a TESLA-340 electron microscope.

Plant material required for SEM was fixed and dehydrated as above. Samples were critical-point dried using carbon dioxide, gold-coated in a CS 100

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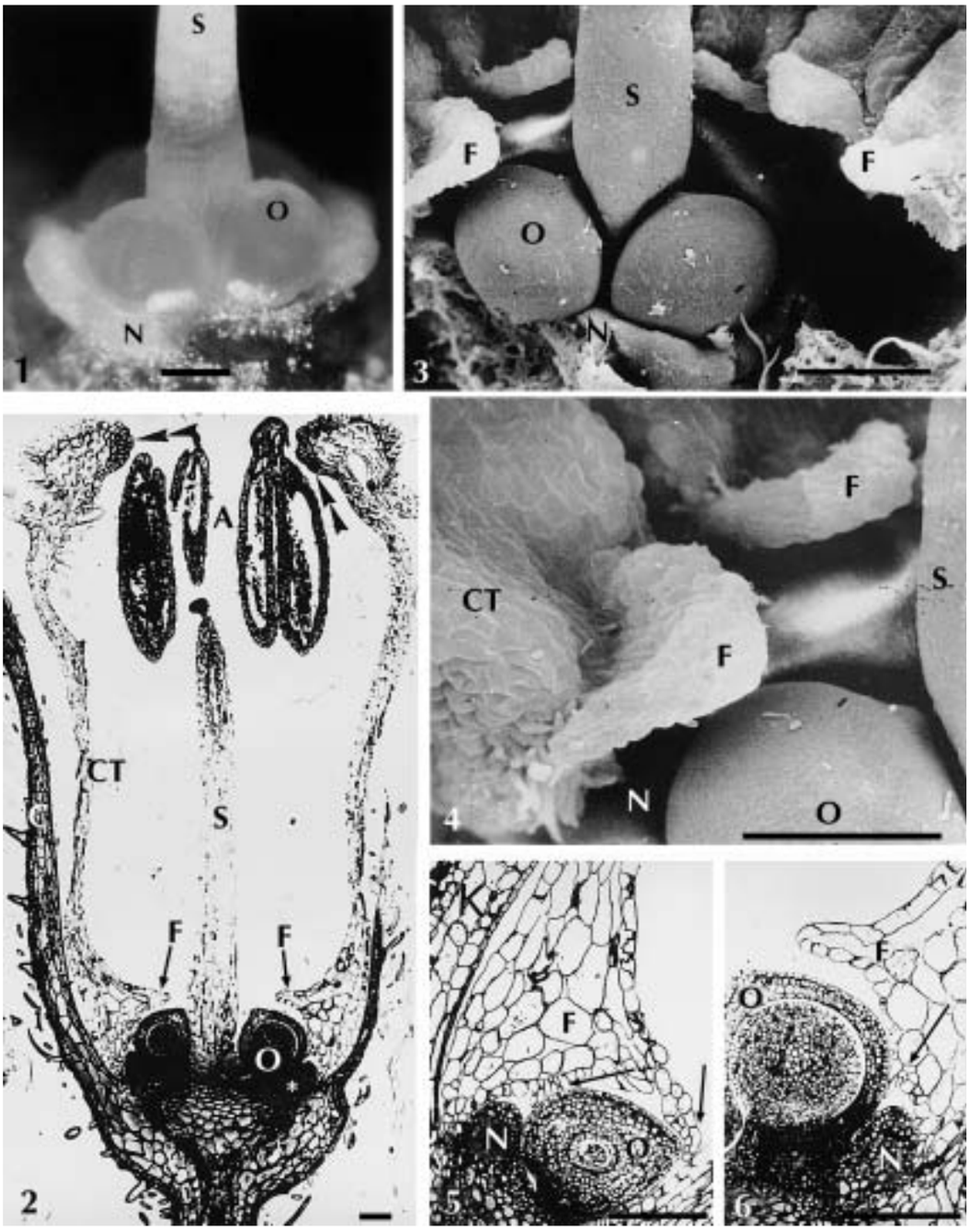

Fig. 1. Portion of *Myosotis sylvatica* flower with nectary (N) at base of four-lobed ovary (O). S – style. **Figs. 2–6.** Fragments of *M. sylvatica* flowers with nectary (N) and corolla tube folds (F). **Fig. 2.** Longitudinal section of flower after removal of corolla lobes; visible scales (double arrowheads) at entrance to corolla tube, nectary (asterisk) and corolla folds (F) above ovary and nectary. **Figs. 3–4.** Portion of flower with ligulate corolla tube folds (F), nectary (N), ovary (O) and style (S). **Figs. 5–6.** Corolla tube folds (F) with epidermis outgrowths (arrows) protecting the nectary (N) and ovary (O). Fig. 2 LM photograph, semithin section; Figs. 3–4 SEM photographs; Figs. 5–6 LM photographs, semithin sections. C – calyx; CT – corolla tube; A – anthers. Bars in all figures = 200 μm.

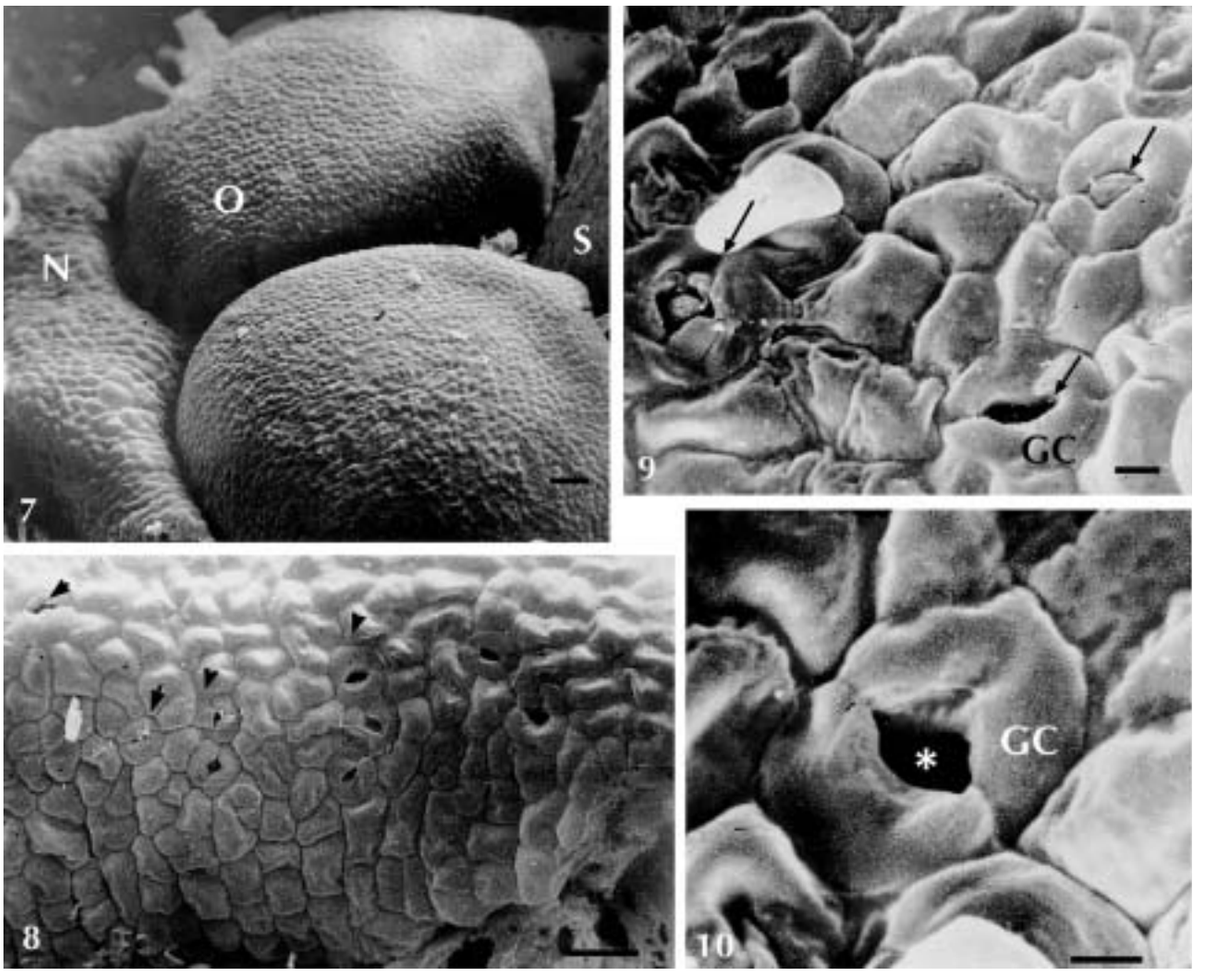

Fig. 7. Discoid nectary (N) below ovary (O), S – style. Bar = 20 μm. **Fig. 8.** Epidermis of nectary with numerous modified stomata (arrowheads) forming stomatal fields. Bar = 20 μm. **Figs. 9–10.** Modified, roundish stomata (arrows) with constantly open porus (asterisk) between guard cells (GC). Bars = 5 μm. Figs. 7–10 SEM photographs.

Sputter Coater and observed with a BS-300 Tesla scanning electron microscope.

RESULTS

FLORAL MORPHOLOGY

Myosotis sylvatica flowers are of radial symmetry, 6–10 mm in diameter. They occur in monochasium inflorescences. The flower has a pentametric perianth, sympetalous corolla, a single whorl of five stamens connected with the corolla tube, and a centrally placed pistil with a four-lobed ovary. The nectary is situated at the base of the ovary and is in the form of an irregular disc (Figs. 1, 7). The corolla is blue, with five yellow nectar guides. The corolla tube is 2 mm long. Five tiny yellow scales narrow the entrance to the corolla tube (Fig. 2). Ten ligulate corolla tube folds are situated above the ovary and nectary, reducing access to nectar (Figs. 2–4). The epidermis of the ligulate folds forms outgrowths adhering to the surface of the ovary and nectary (Figs. 5, 6). The folds are formed of large parenchymatic cells interspersed with large intercellular spaces (Fig. 5). The cell wall of the outgrowths is of uneven thickness. Many vesicles are observed in the cytoplasm (Fig. 14).

Myosotis sylvatica flowers are protogynous. Nectar is secreted in the female and in the male stage of anthesis. In the post-pollination stage the corolla turns violet and the yellow floral guides turn

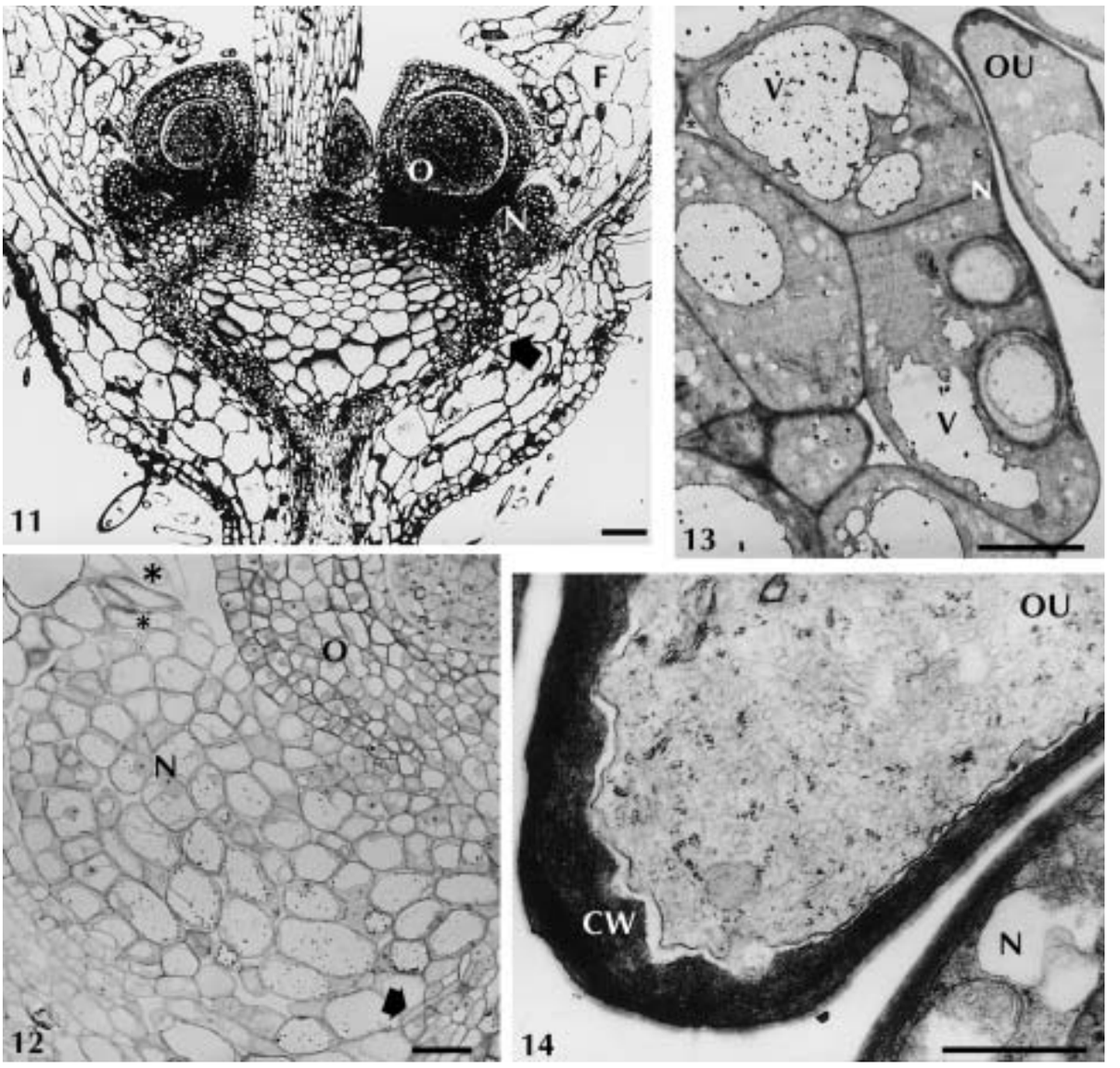

Fig. 11. Lower part of flower. Visible branches of vascular bundles (arrow) in receptacle below nectary (N) and ovary (O). Bar = 100 µm. **Fig. 12.** Vacuolized nectariferous tissue (N) adjoining the ovary (O) and surrounded from the upper side with corolla outgrowths (asterisks). Elements of phloem visible at base of nectary (arrow). Bar = 20 µm. **Fig. 13.** Cells of nectary (N) and corolla outgrowths (OU); visible cells with vacuoles (V) and intercellular spaces of different sizes (asterisks). Bar = 5 µm. **Fig. 14.** Fragments of nectary epidermis cell (N) and of corolla outgrowth cell (OU) with cell wall (CW) of uneven thickness and many vesicles in the cytoplasm. Bar = 1 µm. Figs. 11–12 LM photographs, semithin sections; Figs. 13–14 TEM photographs.

light brown. The flower lasts 4 days. On the last day of blooming the corolla falls away.

STRUCTURE OF THE NECTARY

The nectary of the *Myosotis sylvatica* flower is yellowish green at anthesis. The diameter of the nectary averages 900 µm and its height is 180 µm (n = 12) in flowers from the lower part of the inflorescence.

Nectar is secreted by modified stomata constantly open at anthesis (Figs. 8–10), situated mainly on the abaxial nectary surface adjoining the corolla tube and on the top of the nectary. The roundish stomata differ in size and are irregularly

distributed, forming aggregates of 2–3 each (Fig. 8). They are situated at the level of the neighboring epidermis cells and have no definite orientation. Stomata are anomocytic, because 6–8 epidermal cells adjoin the guard cells (Figs. 9, 10).

The cuticle covering the nectary is thin (0.08 μm) and smooth (Figs. 9, 10). The outer walls of the epidermis cells are thicker (0.75 μm) than the inner walls (0.3–0.4 μm). Both epidermis and glandular parenchyma cells are small (Fig. 11) and isodiametric (Figs. 9, 12). Intercellular spaces of different size are observed in the nectariferous tissue (Fig. 13), which is 10–14 cells deep (Fig. 12). In the actively secreting nectary the epidermis and glandular cells are vacuolized (Figs. 12, 13). Numerous organelles occur in dense cytoplasm: plastids, mitochondria, vesicles of different sizes, and well-developed ER (Figs. 13, 14). Starch grains were found only in the guard cells of the stomata.

Nectaries are supplied by phloem elements observed at the base of glandular tissue (Fig. 12). No vascular elements were visible between the nectariferous cells.

Drops of nectar exuded from the nectary occur first in the narrow space between the nectary and the corolla tube, and next on the upper surface of the corolla tube folds. Hymenopterous and dipterous insects were observed in the flowers of *M. sylvatica*. The most pollinators visiting the flowers were noticed between 10 a.m. and 15 p.m.

DISCUSSION

The discoid nectaries of *Myosotis sylvatica* Hoffm. showed morphology typical of nectariferous tissue, which consists of epidermis with modified stomata and specialized parenchyma (Fahn, 1979). Vogel (1977) classified that type of gland as the mesophyllary type of nectary.

Nectaries with similar histological features have been observed in other species (Fahn, 1979; Dafni et al., 1988; Nepi et al., 1996; Gaffal et al., 1998; Fahn and Shimony, 2001). In this kind of nectary the nectariferous cells secrete nectar into the intercellular spaces. From those spaces, nectar makes its way out of the epidermis through modified stomata (Fahn, 1979; 1988).

The stomata of the *M. sylvatica* nectary are distributed irregularly, only on the top and abaxial surfaces of the nectary, often 2–3 together, forming stomatal fields. Secreted nectar flows through the capillaries between the surface of the nectary and the base of the corolla tube, and then between the corolla folds on their upper part, where it is available to insects. Tiny nectar drops in the flowers of *M. sylvatica* are visible on the corolla folds above the ovary and the nectary. Kugler (1970) found small drops of nectar at the base of the corolla tube also in *M. palustris*. The corolla folds of *M. sylvatica* could facilitate the upward movement of nectar and also protect it from being diluted during rainfall (cells with thick walls). Dafni (1996) mentions unwettable rims in flowers as the plant's adaptation to the impact of rain on the flowers.

The ramifications of vascular bundles supplying the nectary of *M. sylvatica* are situated at the base of the nectariferous tissue. Only phloem elements were found in the bundles. Frei (1955) also failed to observe vascular elements within the secretory tissue of *M. alpestris*, but observed phloem branches enter the glandular tissue of the nectary in other representatives of Boraginaceae (*Borago, Pulmonaria, Echium*).

In the flowers of Boraginaceae taxons, scales may occur at the base of the corolla lobes (Kugler, 1970; Weberling, 1992). Their function is to ensure a proper orientation to insects visiting the flowers during pollination (Kugler, 1970). However, in the literature apparently there are no references to additional outgrowths of the corolla occurring above the nectary such as those observed in *M. sylvatica* in this study. The ultrastructure of the nectary will be investigated.

REFERENCES

DAFNI A. 1996. Autumnal and winter pollination adaptions under Mediterranean conditions. *Bocconea* 5: 171–181.

DAFNI H, LENSKY Y, and FAHN A. 1988. Flower and nectar characteristics of nine species of Labiatae and their influence on honeybee visits. Journ*al of Apicultural Research* 27: 103–114.

FAHN A. 1952. On the structure of floral nectaries. *Botanical Gazette* 113: 464–470.

FAHN A. 1979. Ultrastructure of nectaries in relation nectar secretion. *American Journal of Bot*any 66: 977–985.

FAHN A. 1988. Secretory tissue in vascular plants. *New Phytologist* 108: 229–257.

FAHN A, and SHIMONY C. 2001. Nectary structure and ultrastructure of unisexual flowers of *Ecballium elaterium* (L.) A. Rich. (Cucurbitaceae) and their presumptive pollinators. *Annals of Botany* 87: 27–33.

FREI E. 1955. Die Innervierung der floralen Nektarien dikotyler Pflanzenfamilien. *Berichte der Schweizerischen Botanischen Gesellschaft* 65: 60–114.

GAFFAL KP, HEIMLER W, and EL-GAMMAL S. 1998. The floral nectary of *Digitalis purpurea* L., structure and nectar secretion. *Annals of Bot*any 81: 251–262.

KUGLER H. 1970. *Blütenökologie*. G. Fischer Verlag, Stuttgart.

MAURIZIO A. 1953. Weitere Untersuchungen an Pollenhöschen. *Beihefte Schweiz Bienen-Zeitung* 2: 485–545.

MAURIZIO A, and LOUVEAUX J. 1963. Pollen de plantes melliferes d'Europe, IV. *Pollen et Spores* 5: 213–214.

NEPI M, PACINI E, and WILLEMSE MTM. 1996. Nectary biology of *Cucurbita pepo*: ecophysiological aspects. *Acta Botanica Neerlandica* 45: 41–54.

PROCTOR M, YEO P, and LACK A. 1996. *The natural history of pollination*. Harper Collins Publishers, London.

ROBERTSON AW, and LLOYD DG. 1991. Herkogamy, dichogamy and self-pollination in six species of *Myosotis* (*Boraginaceae*). *Evolutionary Trends in Plants* 5: 53–63.

ROBERTSON AW, and LLOYD D.G. 1993. Rates of pollen deposition and removal in *Myosotis colensoi*. *Functional Ecology* 7: 549–559.

ROBERTSON AW, and MACNAIR MR. 1995. The effect of floral display size on pollination service to individual flower of *Myosotis* and *Mimulus*. *Oikos* 72: 106–114.

VOGEL S. 1977. Nektarien und ihre Ökologische Bedeutung. *Apidologie* 8: 321–335.

WEBERLING F. 1992. *Morphology of flowers and inflorescences*. Cambridge University Press, Cambridge.