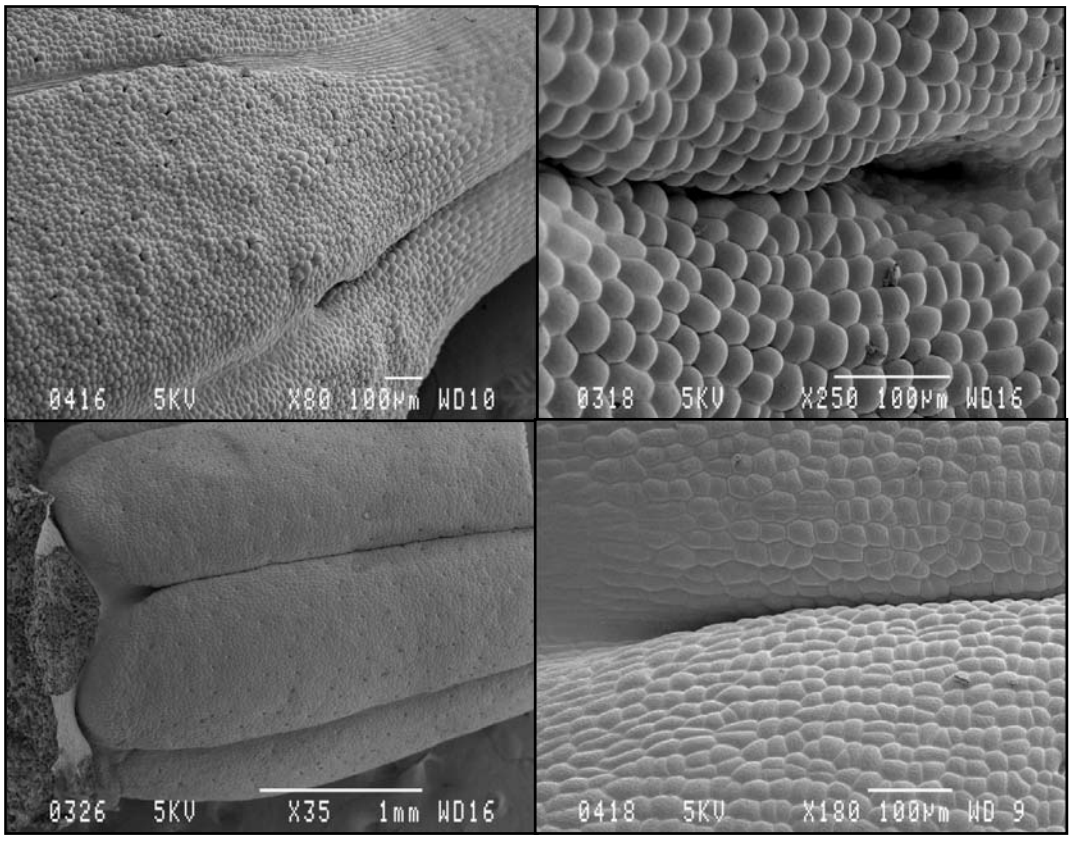
CHAPTER 3

Nectary structure and nectar presentation in *Aloe castanea* and *A. greatheadii* var *davyana* (Asphodelaceae)

M. Nepi[1], H. Human[2], S.W. Nicolson[2], L. Cresti[1], E. Pacini[1]

[1] Department of Environmental Sciences "G. Sarfatti", University of Siena, Via Mattioli 4, 53100 Siena, Italy

[2]Department of Zoology and Entomology, University of Pretoria, Pretoria 0002, South Africa



Paper published in *Plant Systematics and Evolution* **257**: 45-55 (2006)

Abstract

This paper deals with the nectary structure and nectar presentation of two species belonging to different sections of the genus *Aloe*: *A. castanea* (Anguialoe) and *A. greatheadii* var *davyana* (Pictae). The development of the nectary was studied by means of bright field and fluorescence light microscopy and scanning electron microscopy (SEM) in three flower stages (young, intermediate, old). Both species have septal nectaries. In *A. castanea*, a subsidiary tissue, not present in *A. greatheadii* var *davyana*, was found beneath the nectary epithelium. This tissue accumulated starch that was hydrolyzed during secretion. Starch was slightly accumulated around the nectary in *A. greatheadii* var *davyana*. The distribution of chlorophyll in the ovary was also different in the two species. These anatomical differences are not, however, correlated with greater nectar production in *A. castanea*. In this species, the nectary seems to degenerate after secretion, while in *A. greatheadii* var *davyana* no sign of degeneration was observed. Differences in nectar presentation among the two species may account for different pollinators visiting their flowers.

Introduction

Fahn (1979) presented a topographical classification of floral nectaries, indicating nine different types. Among them, the "ovarial nectary" type includes nectaries that are placed in the septal region between adjacent carpels, the so-called septal nectaries or gynopleural nectaries as they have been more recently defined by Smets and Cresens (1988). Gynopleural nectaries are restricted to monocotyledons, where they represent the most common type of floral nectary (Smets et al., 2000). The gynopleural nectary, being a cavity inside the ovary, is not directly exposed to nectar-feeding animals and the site of nectar emission is often different from the site of nectar production (Smets et al., 2000). For this reason we can apply the terminology 'secondary nectar presentation' according to Pacini et al. (2003). Flower morphology and the site of nectar presentation, combined with nectar quantity and composition, are the main factors determining potential pollinators among nectar-feeding animals (Fægri & Van der Pijl, 1979; Baker & Baker, 1983; Proctor et al., 1996). An appropriate positioning of the nectar inside the flower ensures the efficiency of pollination: while exploiting the nectar, the visitor should inevitably contact the reproductive organs.

In this paper we describe the structure of the gynopleural nectaries of *Aloe castanea* (Schönland) and *A. greatheadii* var *davyana* (Schönland) Glen & D.S. Hardy. The genus *Aloe*, family Asphodelaceae, consists of about 350 species occurring across a wide range of habitats in Africa, Madagascar and nearby Comoro Islands, the Middle East, and the Canary Islands. The huge variation in size, length and width of leaves, leaf markings, raceme length and even flower size has led to the division of the genus into 26 sections (Reynolds, 1950; Holland, 1978; Van Wyk & Smith, 1996; Glen & Hardy, 2000). *Aloe greatheadii* var *davyana* belongs to the largest section, Pictae or spotted aloes, and exists either as solitary plants or in large colonies. This aloe produces pink tubular flowers during winter (from June to August) and has a widespread distribution range across the summer rainfall areas of South Africa. The plants grow well on rocky terrain and on grassy plains and are most dense in overgrazed areas (Van Wyk & Smith, 1996; Glen & Hardy, 2000). *Aloe greatheadii* var *davyana* is an extremely important bee plant in South Africa, and beekeepers are known to move their beehives to the aloe fields in winter to make use of the strong pollen and nectar flow (Fletcher & Johannsmeier, 1978).

Aloe castanea belongs to the section Anguialoe. This multistemmed aloe, 2-4 m tall with branched stems, has long curved inflorescences with subsessile orange-brown flowers and abundant nectar. It flowers from July to August and occurs in hot, dry thorny woodland in Mpumalanga and Limpopo Provinces of South Africa and in Swaziland (Van Wyk & Smith, 1996; Glen & Hardy, 2000). Aloes are very important nectar producers in dry habitats, but very little is known about their nectary structure and the manner of nectar presentation to pollinators. Due to of the differences in flower morphology and potential pollinators of *A. castanea* and *A. greatheadii* var *davyana,* we would expect different mechanisms of nectar transport and presentation

Methods

Plant material

Ovaries of *A. castanea* (Schönland) were collected from plants growing in the Pretoria Botanic Garden (Fig. 1) and those of *A. greatheadii* var *davyana* (Schönland) Glen & D.S. Hardy from plants in Roodeplaat Nature Reserve, Gauteng (28º 39'E, 25º 66'S) (Fig. 5). Voucher specimen collection was therefore not necessary for *A. castanea* and *A. greatheadii* var *davyana* was identified in natural habitats by taxonomic experts. Three different flower stages were examined for each species: young flowers with the corolla starting to open but not all anthers dehisced; intermediate flowers with all the anthers dehisced and the corolla completely open; old flowers in which the corolla had started to wilt. Nectar production rate varies with age in a similar manner in the two species: increasing from young to intermediate stages and decreasing in old flowers (Nicolson & Nepi, 2005).

Light microscopy and histochemistry

Ovaries were dissected from flowers under a stereo microscope and fixed in 5 % glutaraldehyde in phosphate buffer (pH 6.9), dehydrated in an ethanol series and embedded in Technovit 7100 (Hereus Kulzer GmbH). A complete series of semi-thin sections (3-5 µm) was obtained with an LKB 8800 microtome. Sections from corresponding parts of the ovary were stained for histochemistry with the following:

a) Toluidine Blue O as general staining (O'Brien & McCully, 1981);
b) PAS (periodic acid/Schiff's reaction) for total insoluble polysaccharides (O'Brien & McCully, 1981);

c) Alcian Blue for pectins (O'Brien & McCully, 1981);
d) IKI (iodine-potassium iodide or Lugol) for starch (Johansen, 1940);
e) Auramine O for cuticle (Heslop-Harrison, 1977);
f) Aniline Blue for callose (Johansen, 1940).
In addition, thin hand-cut sections (20-50 µm) of young ovaries were mounted in distilled water on slides and examined on a Zeiss Axiovert 200 inverted microscope (Carl Zeiss, Götingen, Germany) at 10x magnification for autofluorescence of chloroplasts.

Scanning electron microscopy

Cross and longitudinal sections were made of the ovaries from the three different flower stages of *A. castanea* and *A. greatheadii* var *davyana*. In order to be able to distinguish between top and bottom, we left the upper part of each ovary with 1 mm of style still attached. The material was fixed in 2.5% glutaraldehyde and a sodium phosphate buffer (pH 7.4) for 1 h. Material was rinsed for three times for 10 min each in a sodium phosphate buffer prior to post-fixation with 1% aqueous osmium oxalate for 1 h. Thereafter the material was rinsed with distilled water for twice for 10 min each, and dehydrated in an increasing ethanol series (30, 50, 70, 90 and 100%) for 10 min each. The 100% ethanol was repeated three times before critical point drying in a Polaron critical point drier using carbon dioxide. Material was mounted on SEM stubs, sputter coated with gold and viewed with a JEOL 840 SEM (Tokyo, Japan) at the Laboratory for Microscopy and Microanalysis at the University of Pretoria.

Results

Flower morphology and nectar presentation

Flowers of *A. castanea* form dense inflorescences (Fig. 2). Each flower has an orange-brown, cylindric-campanulate perianth widely opened at the top and generally oriented upward or horizontally. There are six flattened filaments bearing anthers. All the filaments (and the anthers) are exerted from the perianth, the inner ones being longer than the outer ones. The superior ovary is orange (Fig. 3) and bears a long style ending with a tiny stigma positioned at the level of the anthers with longer filaments. Nectar accumulates at the top of the ovary (Fig. 3) filling the space between the filaments. Nectar is pale when just secreted but soon becomes dark reddish-brown (Figs 3, 4). The

pink-orange flowers of *A. greatheadii* var *davyana* form less dense inflorescences (Fig. 6). The orientation of the flowers changes according to their development: from upward as buds to downward just before anthesis, and up again after the end of anthesis and during fruit development (Fig. 6). The perianth is narrower than in *A. castanea* and forms a tube, from which the anthers of the longer filaments are exerted first (Fig. 7). The perianth enlarges at the base of the ovary to form a small bulb (Fig. 7). The disposition of gynoecium and androecium is very similar to *A. castanea*. The superior ovary is green (Fig. 8) and has a long style ending with a tiny stigma, slightly curved. Nectar accumulates around the inferior third of the ovary (Fig. 8), filling the bulb formed by the perianth enlargement. When secretion is particularly abundant, nectar flows along the filaments and the style and may appear at the mouth of the perianth tube where it becomes accessible for bees.

Nectary structure and development

The general anatomy of the nectary is similar in both species. The gynopleural nectaries consist of three clefts located in the septal region between adjacent carpels (Fig. 9). The cavities are lined by secretory epithelium characterised by small cells with dense cytoplasm and large nuclei (Fig. 10). A very thin and irregular cuticle is present on the surface of the epithelium (Fig. 11). Beneath the epithelium in *A. castanea* there is a subsidiary tissue composed of vacuolated cells which are smaller than cells in the other parts of the ovary parenchyma (Fig. 10). This tissue is not clearly evident in *A. greatheadii* var *davyana* and the nectar cavity is extremely reduced (Fig. 12). In this species, the outer tangential walls and the distal part of the radial walls of the epithelium cells appear thicker (Fig. 12) and are intensely stained by PAS (Fig. 13a) and Alcian Blue (Fig. 13b) and have a somewhat “corroded” appearance. In the old flower, the nectar cavity of *A. greatheadii* var *davyana* has almost completely disappeared and the walls described above are thicker and more intensely stained by PAS (Fig. 14) and Alcian Blue (Fig. 15a). In the same walls there is an irregular deposition of callose (Fig. 15b). The modifications of the walls are less evident in *A. castanea,* where there is a reduced thickening in young (Figs 16a, b) and old (Fig. 17) flowers and there is no deposition of callose in the old stage. The epithelial and subsidiary cells of *A. castanea* undergo cytological modification during development, being more vacuolated and with an irregular nuclear shape in the old flower stage (Fig. 18). Some cells in the subsidiary tissue seem to degenerate (Fig. 18). These modifications are not evident in *A.*

greatheadii var *davyana,* where epithelial cells maintain their initial shape even in the old flower stage (Fig. 14).

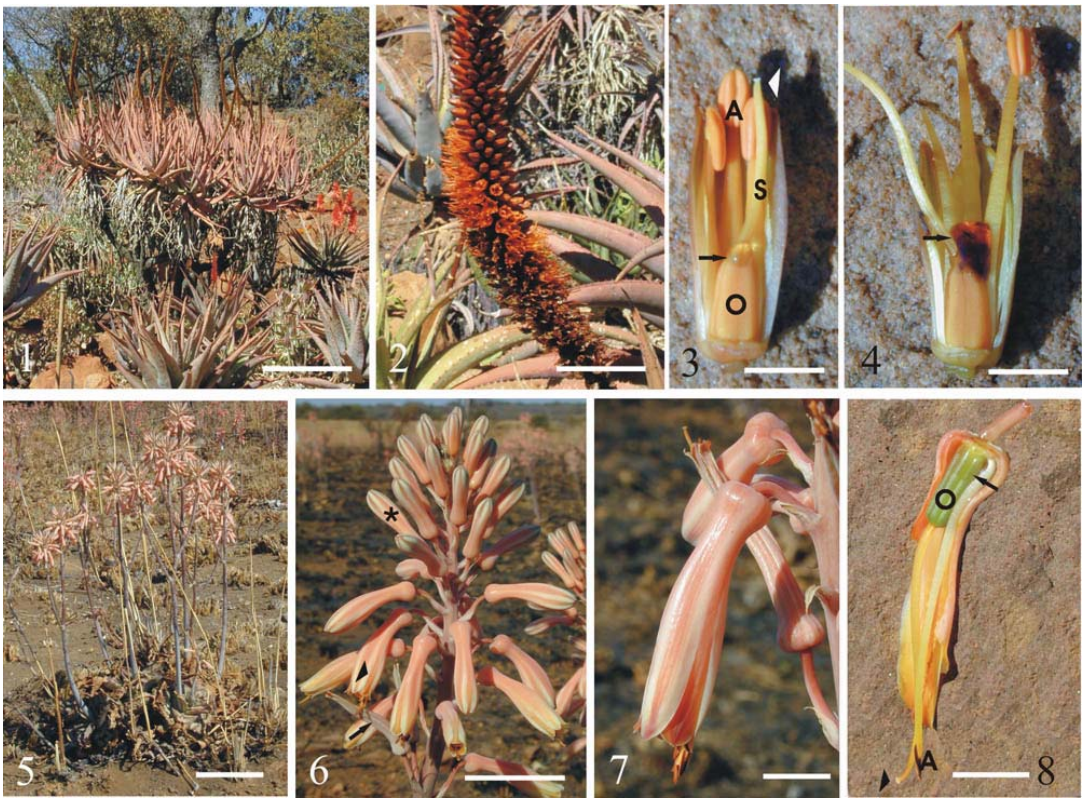


Plate 1

Plate 1. (Fig. 1) A large multi-stemmed plant of *Aloe castanea* in the Pretoria Botanic Garden. Bar = 1 m. **(Fig. 2)** The long curved inflorescence of *Aloe castanea* with densely-packed orange-brown flowers that bend sideways and upwards. Bar = 10 cm **(Fig. 3)** A flower of *Aloe castanea* in the young stage with the corolla and filaments partially removed. The superior ovary (O) is orange and has a long style (S) ending with a tiny stigma (arrowhead). Freshly secreted pale nectar is present at the top of the ovary (arrow). A = anthers. Bar = 0.5 cm. **(Fig. 4)** A flower of *Aloe castanea* in the middle stage with the corolla and filaments partially removed. As secretion proceeds, nectar (arrow) accumulates in the space between the filaments and becomes dark red-brown. Bar = 0.5 cm. **(Fig. 5)** Plants of Aloe *greatheadii* var *davyana* in Roodeplaat Nature Reserve, Gauteng. Bar = 15 cm. **(Fig. 6)** An inflorescence of *Aloe greatheadii* var *davyana*. The pink-orange flowers change in orientation during development. They are in an upward position before anthesis (asterisk), they bend downward just before anthesis (arrowhead) and upward again after the end of anthesis and during fruit development (arrow). Bar = 3 cm. **(Fig. 7)** Close-up of a flower of *A. greatheadii* var *davyana.* At anthesis the longer anthers are exerted from the perianth tube. The perianth is enlarged at its base. Bar = 1.5 cm. **(Fig. 8)** A flower of *Aloe greatheadii* var *davyana* in the middle stage with the corolla and filaments partially removed. The superior ovary is green (O). The long style ends with a tiny slightly curved stigma (arrowhead). A = anthers. Nectar accumulates around the base of the ovary (arrow). Bar = 1.5 cm.

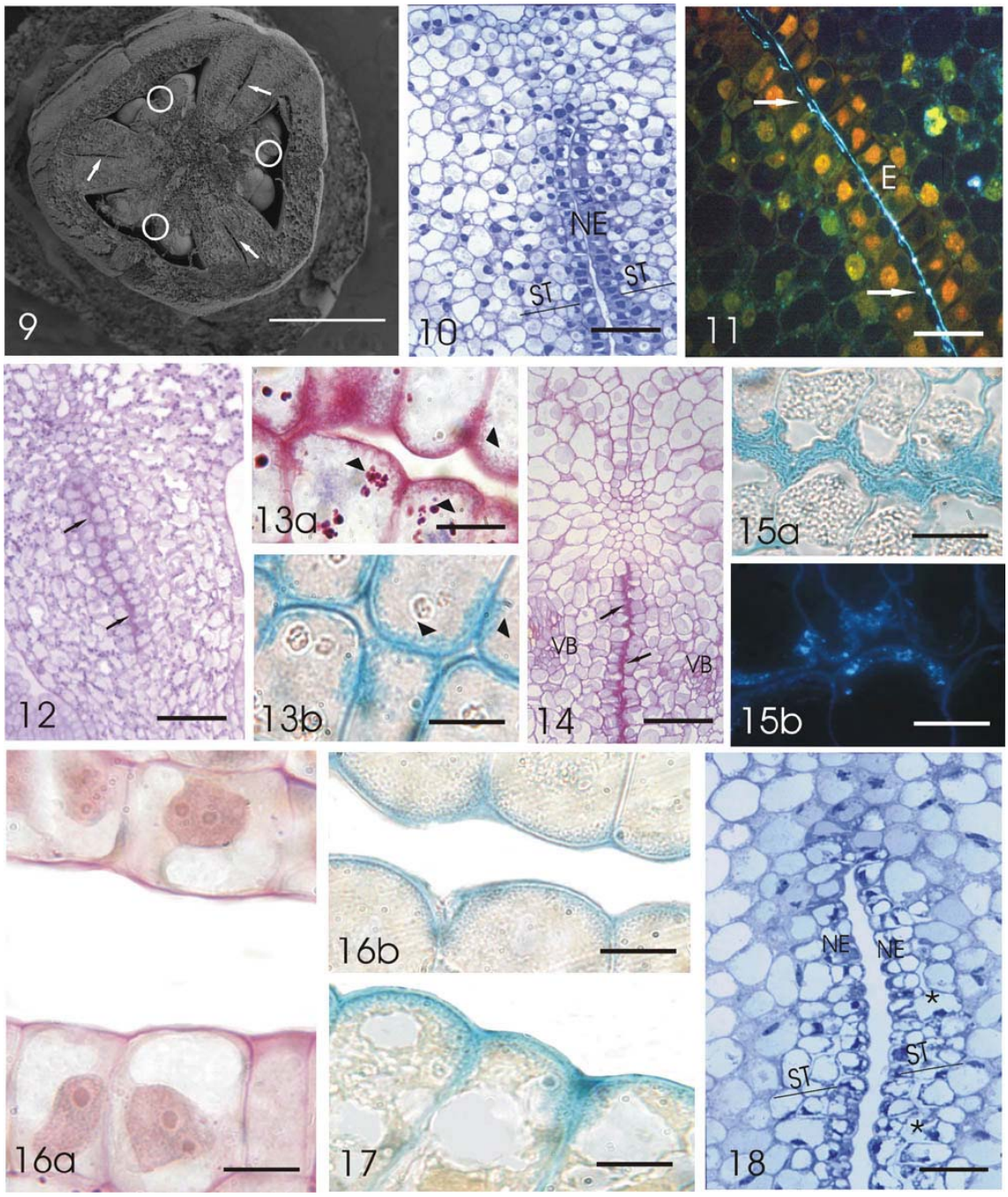

9
10
NE
ST
ST
11
E
12
13a
13b
14
VB
VB
15a
15b
16a
16b
17
18
NE
NE
ST
ST
*
*

Plate 2

Plate 2. (Fig. 9) Scanning electron micrograph of a cross section of the ovary of *A. greatheadii* var *davyana* showing the localization of the three gynopleural nectaries (arrows) alternating with the ovary locules. O = ovules. Bar = 1 mm. **(Fig. 10)** Cross section of the ovary of an *A. castanea* flower in the young stage, stained with Toluidine Blue O. The nectary cavity is lined by an epithelium (NE) made of small cells with dense cytoplasm and relatively large nuclei. A subsidiary tissue (ST) is present around the nectary. Bar = 200 µm. **(Fig. 11)** Cross section of the ovary of an *A. castanea* flower in the young stage, stained with Auramine O. A very thin and irregular cuticle (arrows) is present on the surface of the epithelium. Bar = 100 µm. **(Fig. 12)** Cross section of the ovary of an *A. greatheadii* var *davyana* flower in the young stage, stained with PAS. The nectary cavity is reduced compared to that of *A. castanea* and the subsidiary tissue is not evident. The thick tangential outer walls (arrows) of the epithelial cells are intensely stained by PAS. Bar = 200 µm. **(Fig. 13)** Nectary epithelium cells in the young flower of *A. greatheadii* var *davyana* stained with PAS (**a**) and with Alcian Blue (**b**). The outer tangential walls and the distal part of the radial walls (arrow heads) are thicker than the other walls and have a somewhat corroded appearance. Bar = 30 µm. **(Fig. 14)** Nectary of *A. greatheadii* var *davyana* in the old flower stage stained with PAS. The nectar cavity is occluded and the outer tangential walls of the epithelium cells (arrows) are more densely stained by PAS than in the young stage. VB = vascular bundle. Bar = 200 µm. **(Fig. 15)** Nectary epithelium cells in the old flower of *A. greatheadii* var *davyana* stained with Alcian Blue (**a**) and with Aniline Blue (**b**). The outer tangential walls and the distal part of the radial walls of the epithelium cells appear thicker and more intensely stained by Alcian Blue than in the young stage (Fig 13b). The Aniline Blue reveals an irregular deposition of callose. Bar = 30 µm. **(Fig. 16)** Nectary epithelium cells in the young flower of *A. castanea,* stained with PAS (**a**) and Alcian Blue (**b**). The outer tangential walls and the distal part of the radial walls are less thick and less stained compared to the same stage in *A. greatheadii* var *davyana*. Bar = 15 µm. **(Fig. 17)** Nectary epithelium cells in the old flower of *A. castanea* stained with Alcian Blue. The outer tangential walls and the distal part of the radial walls are less thick and less stained compared to the same stage in *A. greatheadii* var *davyana*. Bar = 15 µm. **(Fig. 18)** Nectary of *A. castanea* in the old flower stained with Toluidine Blue O. The epithelium cells (NE) and the cells of the subsidiary tissue (ST) are more vacuolated than in the young stage. Some cells in the subsidiary tissue seem to degenerate (asterisk). Bar = 100 µm.

Starch is present in both species in the young stages, but with different localisation. In *A. castanea*, starch is present throughout the ovary but at higher concentration in the subsidiary tissue around the nectary (Fig. 19a). In *A. greatheadii* var *davyana,* starch is present mainly in the cortical part of the ovary and in very low quantity around the nectary (Fig. 20a). In both cases starch is completely hydrolysed in the old flower stage (Figs 19b and 20b).

Chlorophyll is present in the ovary of both species, although that of *A. castanea* appears deep orange. In this species chlorophyll has a homogeneous distribution throughout the ovary, being present also in the subsidiary tissue around the nectary (Fig. 21). In *A. greatheadii* var *davyana*, chlorophyll is present in the ovary wall while it is almost absent in the tissue around the nectary (Fig. 22). Vascular bundles containing phloem and xylem were observed around the nectary (see Fig. 14).

Nectar outlet

In *A. castanea,* each nectary cavity has a nectar outlet located just at the base of the style (Fig. 23). It is derived from the merging of an invagination of the cutinised epidermal surface, in continuity with the carpellary suture (Fig. 23), with the apical part of the nectary (Figs 24 and 25). Small cells are present in the vicinity of the merging point (Fig. 24).

In *A. greatheadii* var *davyana,* the carpellary suture is wide at the base of the ovary but it becomes deeper and narrower towards the top of the ovary (Fig. 26 and Figs 27a, b). At two-thirds from the top of the ovary, the invagination of the epidermal surface has tightly connivent margins, except in the inner part where a tubular structure is formed (Fig. 27c). The tubular structure becomes deeper towards the top of the ovary, where it merges with the apical part of the nectary (Fig. 27d). Small cells are present in the inner part of the tubular structure (Fig. 28). Although the tubular structure is in continuity with the outside, this communication is prevented by the presence of the cuticle that occludes the narrow space between the connivent margins of the epidermis (Fig. 29).

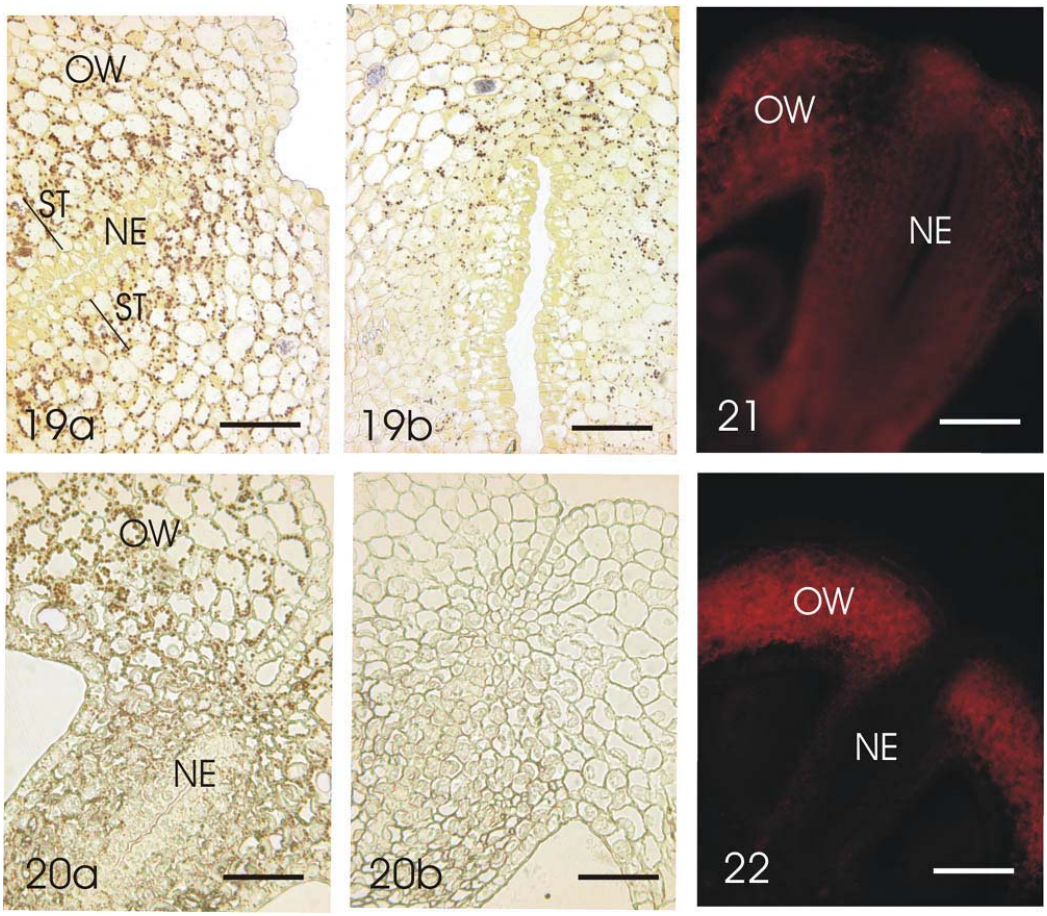


Plate 3

Plate 3. (Fig. 19) Nectary of *A. castanea* in the young (**a**) and old (**b**) flower stained with IKI. In the young flower starch is present in higher concentration in the subsidiary tissue (ST) around the nectary epithelium (NE). Starch is almost completely hydrolysed in the old flower. OW = ovary wall. Bar = 200 µm. **(Fig. 20)** Nectary of *Aloe greatheadii* var *davyana* in the young (**a**) and old (**b**) flower stained with IKI. In the young flower starch is present in higher concentration in the ovary wall (OW). Starch is completely hydrolysed in the old flower. NE = nectary epithelium. Bar = 200 µm. **(Fig. 21)** Chlorophyll autofluorescence in the ovary of *A. castanea*. Chlorophyll has a homogeneous distribution being present also in the subsidiary tissue around the nectary. NE = nectary epithelium; OW = ovary wall. Bar = 300 µm. **(Fig. 22)** Chlorophyll autofluorescence in the ovary of *Aloe greatheadii* var *davyana.* Chlorophyll is present exclusively in the ovary wall (OW). NE = nectary epithelium. Bar = 300 µm.

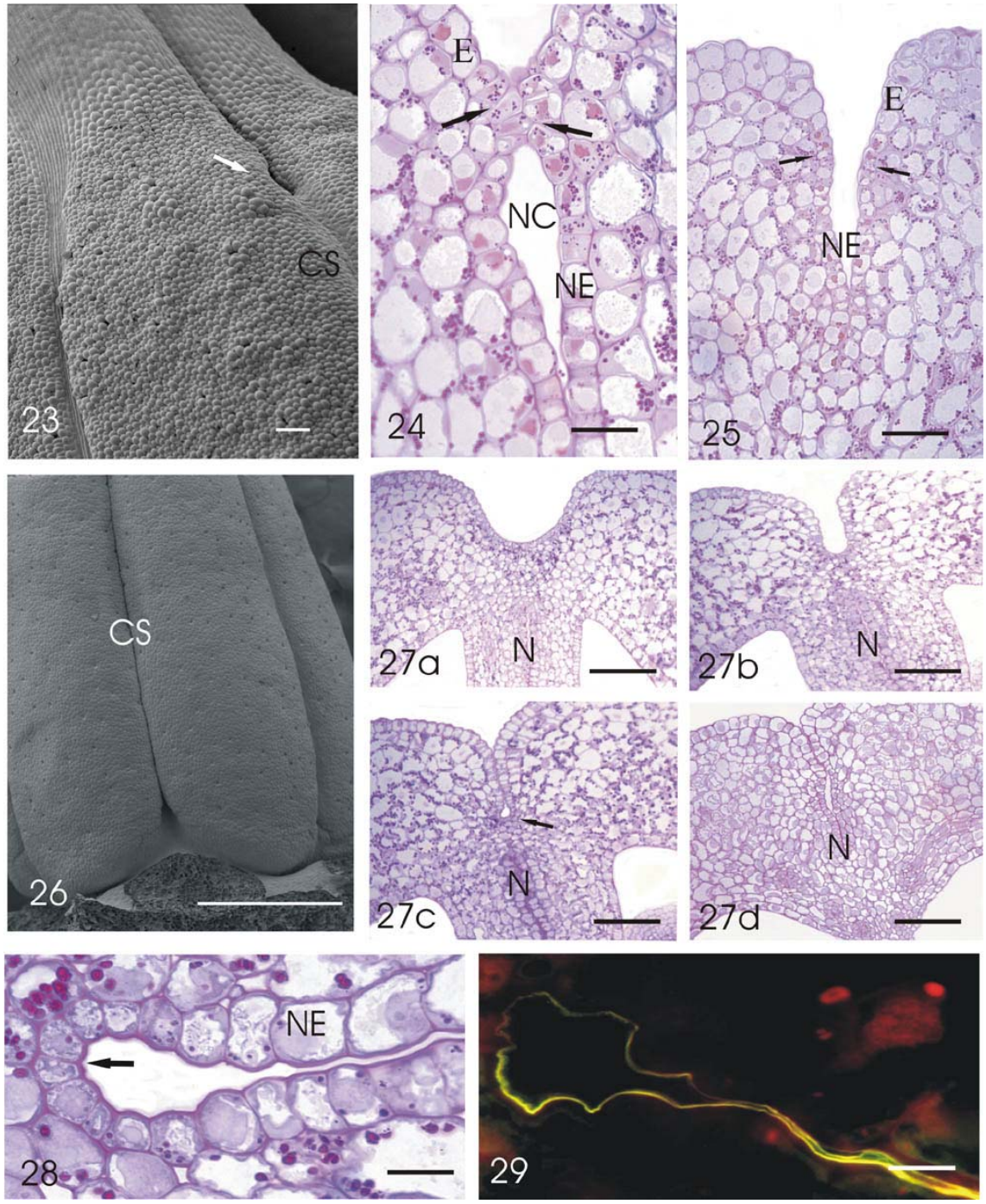


Plate 4

Plate 4. (Fig. 23) Scanning electron micrograph of the ovary of *A. castanea*. The nectary outlet (arrow) is located at the base of the style. CS = carpellary suture. Bar = 100 µm **(Fig. 24)** Cross section of the apical part of the ovary of A. castanea stained with PAS and Coomassie Blue. A few small cells (arrows) are present between the nectary cavity and the epidermal invagination. E = epidermis; NE = nectary epithelium; NC = nectary cavity. Bar = 100 µm. **(Fig. 25)** Cross section of the nectary outlet at the base of the style in *A. castanea*. Small cells (arrows) are present between the nectary epithelium (NE) and the epidermis (E). Bar = 200 µm. **(Fig. 26)** Scanning electron micrograph of the base of the ovary in *A. greatheadii* var *davyana*. The carpellary suture (CS) is wide at the base of the ovary and becomes narrower towards the top of the ovary. Bar = 1 mm. **(Fig. 27) a-d**. Sequential cross sections of the ovary of *A. greatheadii* var *davyana* stained with PAS and Coomassie Blue. **a**. The wide carpellary suture at the base of the ovary becomes narrower and deeper towards the top of the ovary. **b**. At 1/3 of the distance from the top of the ovary, a deep epidermal invagination is present. **c**. This invagination became deeper and with connivent margins at two thirds from the top, forming a tube-like structure (arrow). **d**. This structure merged with the nectary cavity at the top of the ovary. N=nectary. Bar = 400 µm. **(Fig. 28)** Cross section of the deep epidermal invagination in the ovary of *A. greatheadii* var *davyana* stained with PAS and Coomassie Blue. The invagination ends with a tube-like structure with small cells (arrow) in the inner part. Bar = 50 µm. **(Fig. 29)** Cross section of the deep epidermal invagination in the ovary of *A. greatheadii* var *davyana* ending with a tube-like structure stained with PAS and Auramine O. The cuticle occludes the narrow space between the connivent margins of the epidermis. Bar = 50 µm.

Discussion

Nectar anatomy and development

According to Smets et al. (2000), there are two main nectary types in monocotyledons: septal (i.e. persistent) and perigonal (i.e. caducous). In both *Aloe* species typical septal nectaries were found. The morphological characters of these nectaries correspond to the "liliad type" described by Schmid (1985) as "non-labyrinthine distinct septal nectaries" and are considered by this author to be a primitive character in the phylogeny of monocotyledons.

Development of septal nectaries follows two patterns that differ mainly in the fate of the nectary after the secreting phase. A breakdown of the nectary epithelium after secretion was demonstrated in *Musa paradisiaca* female flowers (Fahn & Kotler, 1972); while the transformation of the nectary tissue into parenchyma, by means of elongation of epithelium cells and occlusion of the nectary cavity, has been reported in *Aloe, Gasteria* and *Tillandsia* (Schnepf & Pross, 1976; Cecchi Fiordi & Palandri, 1982). Schnepf and Pross (1976) also demonstrated differentiation of transfer cells in the epithelium of the septal nectaries in some *Aloe* species. A short time before anthesis they form an elaborate system of wall protuberances along their outer walls. In the developing fruit they redifferentiate, lose the wall protuberances, increase in size, and become parenchymatous cells. The redifferentiation of transfer cells was accompanied by the transformation of amyloplasts into chloroplasts. The differentiation of transfer cells in septal nectaries is supposed to be an anatomical mechanism to increase nectar output (Schmid, 1985). According to our observations, the differentiation of epithelial cells into transfer cells most probably occurred in both *Aloe* species, but the transformation of the nectary tissue into parenchyma can be hypothesised only for *A. greatheadii* var *davyana.* The differentiation of thickened outer walls in the epithelium cells was already evident in the young stage in *A. greatheadii* var *davyana* where they have a somewhat "corroded" appearance (see figs 13a, b), as reported by Saunders (1890) for *Kniphofia,* an aspect that can be related to the differentiation of transfer cells (Schmid, 1985). In *A. greatheadii* var *davyana*, the elongation of epithelial cells is not evident, and moreover the nectar cavity is completely occluded in the old stage. The deposition of callose in the thickened outer walls signalled the end of secretion activity, as reported also by Schnepf and Pross (1976). In *A. castanea*, the vacuolation and elongation of

epithelial cells is evident in old flowers but the nectar cavity is still present; in addition, the thickening of the epithelial cells' outer walls is reduced in comparison to *A. greatheadii* var *davyana* and there is no deposition of callose in those walls.

The localisation of chlorophyll and starch storage sites overlap in both species. In *A. greatheadii* var *davyana* both chlorophyll and starch are concentrated in the ovarian wall. In *A. castanea* chlorophyll is also present around the nectary, where an increased starch accumulation was observed. These differences between *A. greatheadii* var *davyana* and *A. castanea* are related to the different extent of the subsidiary glandular tissue underlying the epithelium cells. This tissue is more developed in *A. castanea,* and evidently photosynthesising and able to store starch. Different extents of the subsidiary tissue were also observed in different species of *Tillandsia* (Cecchi Fiordi & Palandri, 1982) and were related to differences in nectar production. In both *Aloe* species almost all the starch was hydrolysed in the old flower stage, suggesting a correlation between nectar production and starch hydrolysis, as observed for other species secreting copious quantities of nectar (Nepi et al., 1996; Durkee et al., 1981; Pacini et al., 2003). The greater quantity of starch around the nectary does not, however, result in greater sugar production in *A. castanea* (mean volume per flower 44.6 µl, concentration 16.0%) (Nicolson & Nepi, 2005) compared to *A. greatheadii* var *davyana* (mean volume per flower 30.7 µl, concentration 23.5%) (Chapter 4).

Nectar presentation and pollinators

Just as pollen has primary and secondary presentation (Faegri & van der Pijl, 1979), the same was proposed for nectar by Pacini et al. (2003). The presentation is primary when the site of nectar production and the site of nectar emission are the same - the more common situation. When these sites are different the term secondary presentation is used. In this case nectar flows from the nectary and collects in another part of the flower. As in all plants having septal nectaries, *Aloe* species have secondary nectar presentation (Dauman, 1970; Smets et al., 2000). Nonetheless, nectar presentation is different in the two species we studied. *A. castanea* has primary nectar outlets located at the base of the style and nectar accumulates at the top of the ovary, sometimes filling the corolla tube. The system of secondary nectar presentation is more complicated in the case of *A. greatheadii* var *davyana,* where secondary drainage through a capillary duct is present and nectar is accumulated at the base of the ovary in a bulb formed by an

enlargement of the corolla. In *A. greatheadii* var *davyana* a primary nectar outlet is present at the top of the ovary, from where the nectar is transported by means of capillarity through the tubular structure formed by deep invagination of the epidermis. When this deep invagination enlarges, about at one third of the ovary length from its base, the nectar may flow into the bulb through a secondary outlet. These kinds of nectar ducts were reviewed by Vogel (1998) and were described in plants with septal nectaries or in plants where nectar accumulates in spurs or other narrow tubular containers. The ducts that we found in *A. greatheadii* var *davyana* are very similar, from a morphological point of view, to those described in *Milla biflora* (Alliaceae) (Vogel, 1998) although they are longer in the latter species.

Among aloes, the bulb at the base of the corolla is a common feature in the section Pictae (Glen & Hardy, 2000), and species belonging to this section probably have the same nectar presentation as described for *A. greatheadii* var *davyana*.

Because nectar composition is remarkably constant in species of *Aloe* (sucrose is almost absent and there are almost equal amounts of glucose and fructose; van Wyk et al., 1993), the flower morphology and secondary presentation of nectar, which affect nectar availability, may be important to potential animal visitors. According to our observations, honeybees collect only pollen from *A. castanea,* ignoring the very dilute nectar, but collect both pollen and nectar from *A. greatheadii* var *davyana.* Bees are probably effective pollinators in both cases. Bird visitors to *A. castanea* include sunbirds and larger, less specialised passerines while only sunbirds have been observed probing the tubular flowers of *A. greatheadii* var *davyana*.

Acknowledgements

We thank the Pretoria Botanic Garden and Roodeplaat Nature Reserve for permission to work on *Aloe castanea* and *A. greatheadii* var *davyana* respectively. Personnel at the Laboratory for Microscopy and Microanalysis, University of Pretoria, gave assistance in SEM. Financial support was provided by the South African National Research Foundation and the Italian Ministry of Foreign Affairs (General Direction for Cultural Promotion and Cooperation) in the framework of the Italy/South Africa Science and Technology Agreement (2001-2004).

References

Baker, H.G. & Baker, I. (1983) Chemical constituents of nectar in relation to pollination mechanisms and phylogeny. In Nitecki M.H. (Ed), *Biochemical aspects of evolutionary biology*. pp. 131-171, University of Chicago Press, Chicago.

Cecchi Fiordi, A. & Palandri, M.R. (1982) Anatomic and ultrastructural study of the septal nectary in some *Tillandsia* (Bromeliaceae) species. *Caryologia* **35**: 477-489.

Dauman, E. (1970) Das Blütennektarium der Monocotyledonen unter besonderer Berücksichtigung seiner systematischen und phylogenetischen Bedeutung. *Feddes Repertorium* **80**: 463-590.

Durkee, L.T., Gaal, D.J. & Reisner, W.H. (1981) The floral and extrafloral nectaries of *Passiflora*. I. The floral nectary. *American Journal of Botany* **68**: 453-462.

Fægri, K. & van der Pijl, L. (1979) *The principles of pollination ecology*. 3rd revised edition. Pergamon Press, Oxford.

Fahn, A. (1979) *Secretory tissues in plants*. Academic Press, London.

Fahn, A. & Kotler, M. (1972) The development of the flower and nectary of the dwarf cavendish banana. In Murty, Y.S., Johri, B.M., Moham Ram, H.Y., Varghese, T.M. (Eds.), *Advances in Plant Morphology* (**V**. Puri Commemoration Volume). pp. 153-169, Meerut, India.

Fletcher, D.J.C. & Johannsmeier, M.F. (1978) The status of beekeeping in South Africa. *South African Bee Journal* **50**: 5-20.

Glen, H.F. & Hardy, D.S. (2000) Fascicle 1: Aloaceae (First part): *Aloe*. In Germishuizen, G. (Ed), *Flora of Southern Africa* **5,** National Botanical Institute, South Africa.

Heslop-Harrison, Y. (1977) The pollen stigma interaction: pollen tube penetration in *Crocus*. *Annals of Botany* **41**: 913-922.

Holland, G. (1978) An evolutionary biogeography of the genus *Aloe*. *Journal of Biogeography* **5**: 213-226.

Johansen, D.A. (1940) *Plant microtechnique*. McGraw-Hill, New York.

Nepi, M., Ciampolini, F. & Pacini, E. (1996) Development and ultrastructure of *Cucurbita pepo* nectaries of male flowers. *Annals of Botany* **78**: 95-104.

Nicolson, S.W. & Nepi, M. (2005). Dilute nectar in dry atmospheres: nectar secretion patterns in *Aloe castanea* (Asphodelaceae). *International Journal of Plant Sciences* 166: 227-233.

O'Brien, T.P., McCully, M.E. (1981) *The study of plant structure - principles and selected methods*. Termarcarphi Pty, Melbourne.

Pacini, E., Nepi, M. & Vesprini, J.L. (2003) Nectar biodiversity: a short review. *Plant Systematics and Evolution* 238: 7-21.

Proctor, M., Yeo, P. & Lack, A. (1996) *The natural history of pollination*. Harper Collins Publishers, London.

Reynolds, G.W. (1950) *The aloes of South Africa*. Aloes of South Africa Book Fund, Cape Town.

Saunders, E.R. (1890) On the structure and function of the septal glands in *Kniphofia*. *Annals of Botany* 5: 11-25.

Schmid, R. (1985) Functional interpretations of the morphology and anatomy of septal nectaries. *Acta Botanica Neerlandica* 34: 125-128.

Schnepf, E. & Pross, E. (1976) Differentiation and redifferentiation of a transfer cell: development of septal nectaries of *Aloe* and *Gasteria*. *Protoplasma* 89: 105-115.

Smets, E.F. & Cresens, E.M. (1988) Types of floral nectaries and the concept of 'character' and 'character state' – a reconsideration. *Acta Botanica Neerlandica* 37: 121-128.

Smets, E.F., Ronse Decraene, L-P., Caris, P. & Rudall, P.J. (2000) Floral nectaries in monocotyledons. In Wilson, K.L., Morrison, D.A. (Eds.) *Monocots: Systematics and Evolution* pp. 230-240, CSIRO, Melbourne.

Van Wyk, B-E. & Smith, G. (1996) *Guide to Aloes of South Africa*. Briza publications, Pretoria.

Van Wyk, B-E., Whitehead, C.S., Glen, H.F., Hardy, D.S., van Jaarsveld, E.J. & Smith, G. (1993) Nectar sugar composition in the subfamily Alooideae (Asphodelaceae). *Biochemical Systematics and Ecology* 21: 249-253.

Vogel, S. (1998) Remarkable nectaries: structure, ecology, organophyletic perspectives III. Nectar ducts. *Flora* 193: 113-131.