Chapter 3

NECTARY STRUCTURE AND ULTRASTRUCTURE

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1 INTRODUCTION

It is easy to define nectaries from a functional point of view: they are plant-secreting structures that produce nectar, but it is difficult to provide a general definition. From the anatomical point of view nectaries vary widely in ontogeny, morphology, and structure (Fahn, 1979a, 1988; Durkee, 1983; Smets et al., 2000), both between species and within species, depending on flower sexual expression or flower morph in heterostylous and heteroantheric species (Nepi at al., 1996; Küchmeister et al., 1997; Fahn & Shimony, 2001; Pacini et al., 2003). Intraspecific morphological differences exist between flowers of the same plant and between plants of the same species with different ploidy (Davis et al., 1996), and morphological characters may be affected by environmental conditions such as water availability. Petanidou et al. (2000) found that nectary structure in Lamiaceae species in a Mediterranean shrub community was largely shaped by phylogenetic and climate constraints. In the course of the flowering season (spring–summer) stomatal opening and nectary size decreased, thus minimizing nectar flow at a time when water was scarce. They hypothesized that very concentrated nectar was secreted via large modified stomata, whereas cuticular secretion was mainly encountered in species with very thin nectars. Petanidou (2007) speculates that the frequency of species with stomatal nectar secretion should be much higher in hot and arid climates like the Mediterranean and deserts than in temperate ones.

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Sometimes the morphological characters of nectaries seem to be correlated with the quantity of the nectar secreted but not its quality. For example, nectar volume depends positively on the volume of nectariferous tissue (Petanidou et al., 2000). Davis and Vogel (2005) noted that in the polymorphic flowers of *Linaria genistifolia* (Scrophulariaceae), where nectar flows from the nectary into a spur, multi-spurred flowers had larger nectaries that produced a greater volume of nectar than single-spurred and spurless flowers. The volume of secreted nectar does not seem to be correlated with the number of nectarostomata (Petanidou et al., 2000; Teuber et al., 1980; Davis & Gunning, 1991) because not all stomata secrete nectar (Gaffal et al., 1988); however, the opposite is true in Bignoniaceae (Galetto, 1995).

Regardless of this enormous variability, Schmid (1988) defines the nectary as "a more or less localized, multicellular glandular structure that occurs on vegetative or reproductive organs and that regularly secretes nectar, a sweet solution containing mainly sugars and generally serving as a reward for pollinators or for protectors (e.g., ants) against herbivores, or, in carnivorous plants, as a lure for animal prey".

Although in some cases the nectary may be an organ (e.g., the rudimentary carpellodia of staminate flowers of *Buxus*; see Schmid, 1988 and references therein), it is commonly only part of an organ and Schmid's definition can be applied correctly when the nectary is conspicuous, continuous, and occupies a well-defined area. Problems may arise when there are small discontinuous nectar-secreting structures scattered over a large area. Vogel (1998a) termed such small secreting structures as nectarioles, and examples have been found among floral and extrafloral nectariferous organs of *Peperomia* (Piperaceae), *Cabomba* (Cabombaceae), *Sarracenia* (Sarraceniaceae), *Cephalotus* (Cephalotaceae), *Chimonanthus* (Calycanthaceae), *Aristolochia* (Aristolochiaceae). In such cases it is unclear whether the term nectary refers to the individual nectar-secreting areas or to all of them as a whole. The term nectarium, introduced by Linnaeus (1735) and used also by Davis et al. (1998) for the complex nectary of Brassicaceae, can be used to describe all separated nectaries in a flower, whereas nectary represents the single unit (see also Bernardello, 2007, Chapter 2 in this volume).

Nectaries may be located at surface level in the organ bearing them, form an outgrowth on the organ, or be concealed deep within the organ (e.g., the septal or gynopleural nectaries of monocotyledons). Unlike other floral structures, the relative positions of which are conserved throughout the angiosperms, the nectary is not located in the same position in all plants (Fahn, 1979a). From the ecological point of view, the diversity in nectary location

is due to the diversity of pollinators and their foraging behaviour. Baum et al. (2001) recently discovered the molecular basis for such great variability: the nectary is independent of the ABC floral homeotic genes that are responsible for floral organ identity specification according to their position. Thus the nectary is potentially "free" to move about the flower during evolution in response to selection imposed by interactions with pollinators.

2 NECTARY STRUCTURE AND ULTRASTRUCTURE

According to Fahn (1979a, 1988, 2000), the nectary is made up of a tissue called **nectariferous tissue**, which consists of an **epidermis** usually overlying a **specialized parenchymatous tissue** (Fig. 1). Durkee (1983) used the terms **secretory tissue** or **glandular tissue** as synonymous to Fahn's **parenchymatous tissue,** but also introduced the term **subglandular** or **nonglandular parenchyma** (also known as ground parenchyma) to describe one or more cell layers that separate the secretory tissue from the vascular bundle (Fig. 1). Merging these two slightly different definitions, I propose the following three terms to describe the general anatomy of the nectary:

- **Nectary epidermis**.
- **Nectary parenchyma** to indicate the layer(s) of small cells with densely staining cytoplasm generally present beneath the epidermis, corresponding to Fahn's specialized parenchymatous tissue.
- **Subnectary parenchyma**, made up generally by larger cells, more loosely packed than those of the nectary parenchyma, corresponding to Durkee's subglandular parenchyma (Fig. 1).

Vascular bundles may be found in the nectary or subnectary parenchyma. With the proposed definitions it is clear that the epidermis and the nectary parenchyma are the tissues involved in nectar production and secretion—thus constituting the functional unit—while the subnectary parenchyma is not directly involved in nectar production, but may have some functions related to nectar production.

All components of the anatomical structure described above are not always recognizable. This is why Zimmermann (1932) distinguished structural (i.e., nectaries with a defined structure) and non-structural nectaries (i.e., without any special differentiated nectariferous structure). Examples of non-structural nectaries are more frequent among extrafloral nectaries (Fahn, 1979a and references therein) than among floral ones (Bernardello, 2007).

Fahn (1979a)	Durkee (1983)	Nepi (this chapter)	
epidermis	epidermis	epidermis	directly involved in nectar production and secretion
parenchymatous tissue with vascular bundles	secretory tissue	nectary parenchyma	
	sub-glandular parenchyma with vascular bundles	sub-nectary parenchyma with vascular bundles	not directly involved in nectar production

Figure 1. Anatomical organization of the nectary according to Fahn (1979a) and Durkee (1983), and that proposed in the present chapter.

On the other hand, in some species the anatomical structure of the nectary can be recognized, but the nectary does not produce nectar. This is the case of the so-called vestigial nectaries found in some Bignoniaceae (*Catalpa, Clytostoma*, *Cydista*, *Phryganocydia*) (Rivera, 2000 and references therein). The lack of a functional nectary has been associated with pollination by deception in Bignoniaceae (Gentry, 1980).

Before discussing the structure of the different components of the nectary, it is necessary to give some definitions and to clarify differences between the terms nectar production, nectar secretion, and nectar release. Nectar production is sometimes considered synonymous with nectar secretion. In my opinion the two terms are different. Nectar production is a phenomenon related to the nectary as a whole. It comprises different events (sugar unloading from the vascular bundle, transport of molecules into the nectary tissue, transformation of molecules, nectar release from the nectary) leading to nectar release (or exudation) from the nectary. Nectar secretion refers to the release of nectar from the protoplasm of the nectary parenchyma cells, thus it describes a phenomenon at the cellular level.

2.1 Epidermis

Epidermal cells are generally smaller than parenchyma cells; they are polyhedric and may have an anticlinal orientation. The vacuole is generally

bigger than in parenchyma cells. Plastids of epidermal cells do not usually store starch (Razem & Davis, 1999), except when a very high rate of nectar secretion is required, as in *Passiflora* sp. (Durkee et al., 1981), *Rosmarinus officinalis* (Zer & Fahn, 1992), and *Cucurbita pepo* (Nepi et al., 1996).

When nectar secretion does not occur through stomata, the epidermis itself is involved in the secretion process via epidermal secreting cells or secreting trichomes. Though this has not yet been demonstrated, the two manners of secretion are not mutually exclusive and may take place contemporaneously (Nepi et al., 2001). In certain plants, the outer walls of the epidermal cells involved in nectar secretion have wall ingrowths (Schnepf & Pross, 1976; Fahn, 1979a; Fahn & Benouaiche, 1979 and references therein; Davis et al., 1988) and are regarded as transfer cells (Pate & Gunning, 1972). Wall protuberances are thought to aid eccrine secretion of individual molecules and are seldom found in systems where secretion by vesicles (granulocrine secretion) has been suggested (Kronestedt-Robards & Robards, 1991).

The anatomical differences in the structure of nectary epidermis concern: cuticle structure and patterning, the presence/absence and structure of secreting trichomes, the presence/absence of stomata (Table 1).

A continuous cuticle is generally present on the surface of the nectary epidermis, although it may be thinner than on the areas adjacent to the nectary (Gaffal et al., 1998 and references therein) or discontinuous as in septal nectaries. A cuticular lining of intercellular spaces in the more peripheral nectary parenchyma and the substomatal chamber has been reported in a number of species (Rachmilevitz & Fahn, 1973; Davis et al., 1988; Maldonado & Otegui, 1997; Razem & Davis, 1999 and references therein) and may enhance nectar movement once in the apoplast.

The patterning, thickness, and permeability of the nectary cuticle vary widely. In the case of nectary trichomes, the cuticle covering the secreting cell seems to be completely impermeable and the nectar accumulates in a subcuticular space formed by separation of the cuticle from the epidermis (Fig. 2). As secretion proceeds, the cuticle stretches and becomes very thin. It has not been determined whether the nectar is released when the cuticle breaks or whether thin areas of the stretched cuticle become permeable to nectar.

Table 1. Main structural features of selected nectaries, both floral and extrafloral. Starch is indicated in arbitrary units according to its abundance in the nectary parenchyma. – = starch absent; + = few starch grains; ++ = many starch grains; +++ = very abundant starch grains.

Species	Nectary type	Anatomy	Starch	Epidermis and parenchyma plastids	Vascular-ization	Nectar secretion	Nectar release	References
Dicotyledons								
Aptenia cordifolia (Aizoaceae)	F	e+p	+	am	nv	e	cp	Meyberg & Kristen, 1981
Arabidopsis thaliana (Brassicaceae)	F	e+p	+	ch	ph		s	Baum et al., 2001
Brassica napus (Brassicaceae)	F	e+p	+	ch	ph	e	s	Davis et al., 1986
Capparis retusa (Brassicaceae)	F	e+p			ph, xy			Di Sapio et al., 2001
Chamelaucium uncinatum (Myrtaceae)	F	e+p				g	s	O'Brien, 1996
Chamelaucium uncinatum	EF	e+p	+	ch		g	cp, s	O'Brien, 1996
Cucurbita pepo (Cucurbitaceae)	F	e+p	+++	am	ph	e	s	Nepi et al., 1996
Digitalis purpurea (Scrophulariaceae)	F	e+p	++	am	ph		s	Gaffal et al., 1998
Ecballium elaterium (Cucurbitaceae)	F	e+p	++	ch	ph, xy		s	Fahn & Shimony, 2001
Eccremocarpus scaber (Bignoniaceae)	F	e+p	+++	am	ph		s	Belmonte et al., 1994
Echinacea purpurea (Asteraceae)	F	e+p	–	p-ob	ph	e	s	Wist & Davis, 2006

Species	Nectary type	Anatomy	Starch	Epidermis and parenchyma plastids	Vascular-ization	Nectar secretion	Nectar release	References
Euphorbia neriifolia (Euphorbiaceae)	EF	e+p	+	ch	ph, xy	g	bc	Arumugasamy et al., 1990b
Glycine max (Fabaceae)	F	e+p	+	ch	ph	ho		Horner et al., 2003
Hibiscus rosa-sinensis (Malvaceae)	F	t, pl, lin	+	am	ph, xy		bc	Sawidis, 1998
Myosotis sylvatica (Boraginaceae)	F	e+p			ph		s	Weryzsko-Chmielewska, 2003
Passiflora spp. (Passifloraceae)	F	e+p	+++	am	ph, xy	e	s	Durkee et al., 1981
Passiflora spp.	EF	e+p	+	ch	ph	e	bc	Durkee, 1982
Pisum sativum (Fabaceae)	F	e+p	+	ch	ph	e	s	Razem & Davis, 1999
Ricinus communis (Euphorbiaceae)	EF	e+p	+	ch	ph, xy	e	bc	Baker et al., 1978
Rosmarinus officinalis (Lamiaceae)	F	e+p	+++	am	ph	e	s	Zer & Fahn, 1992
Sambucus nigra (Caprifoliaceae)	EF	e+p		ch	ph	g	lc	Fahn, 1987
Sanango racemosum (Buddlejaceae)	F	e+p	+++	am	ph		s	Maldonado & Otegui, 1997
Solanum stramonifolium (Solanaceae)	EF	e+p	+	ch	ph, xy		s	Falcão et al., 2003
Tabebuia serratifolia (Bignoniaceae)	F	e+p	+++	am	ph		s	Vinoth & Yash, 1992
Turnera ulmifolia (Passifloraceae)	F	indefinite	–		nv	ho		Elias et al., 1975

Species	Nectary type	Anatomy	Starch	Epidermis and parenchyma plastids	Vascular-ization	Nectar secretion	Nectar release	References
Turnera ulmifolia	EF	e+p	–		ph, xy		bc	Elias et al., 1975
Vicia faba (Fabaceae)	F	e+p	+	ch	ph	g	s, cm	Davis et al., 1988
Vicia faba	EF	t, pl, cap	+	ch	ph, xy	g	pc	Davis et al., 1988
Monocotyledons								
Gymnadenia conopsea (Orchidaceae)	F	t, pl, lin	–	pl-ob		g	cp	Stpiczyńska & Matusiewicz, 2001
Hexisea imbricata (Orchidaceae)	F	e+p	+	ch	ph, xy	g	cp	Stpiczyńska et al., 2005a
Limodorum abortivum (Orchidaceae)	F	e+p	++	am		g	cd	Figueiredo & Pais, 1992
Maxillaria coccinea (Orchidaceae)	F	e+p	–	ch	ph, xy	e	cp	Stpiczyńska et al., 2003
Musa paradisiaca (Musaceae)	F	s	++	am		g		Fahn & Benouaiche, 1979
Platanthera bifolia (Orchidaceae)	F	t, unicel	+	ch	ph, xy	g	bc	Stpiczyńska, 1997
Platanthera chlorantha (Orchidaceae)	F	t, unicel	+	p-ob		g	cm	Stpiczyńska et al., 2005b

Nectary type: EF = extrafloral nectary; F = floral nectary.
Anatomy: e = epidermis; p = parenchyma; s = septal; t = trichomes; pl = pluricellular; unicel = unicellular; cap =capitate; lin = linear.
Plastids: ch = chloroplasts; am = amyloplasts; pl-ob = plastids with osmiophilic bodies.
Vascularization: nv = no vascularization; ph = phloem; xy = xylem.
Nectar secretion: ho = holocrine; e = eccrine; g = granulocrine.
Nectar release: bc = breaking cuticle; cd = cuticle disruption; cm = cuticle microchannels; cp = cuticle permeation; lc = lysigenous cavity; pc = pores in cuticle; s = stomata.

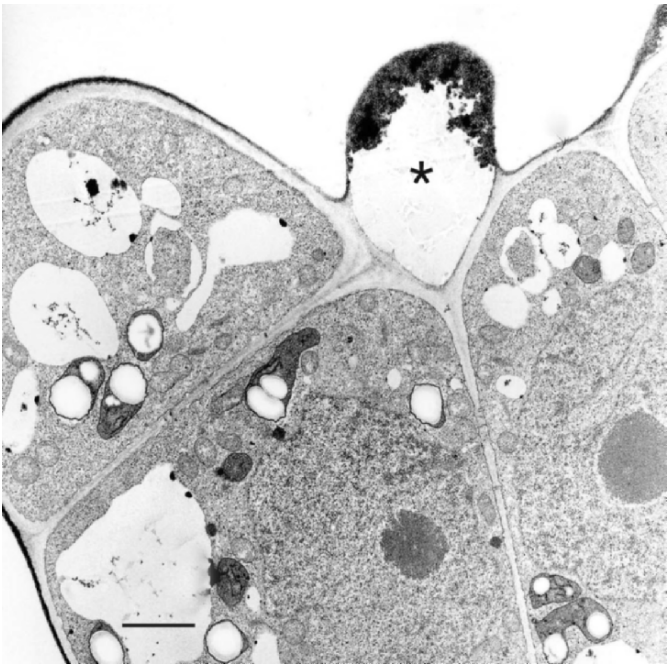

Figure 2. A capitate trichome of the *Cyclanthera pedata* (Cucurbitaceae) nectary at the beginning of nectar secretion. Nectar (*asterisk*) accumulates in a subcuticular space, stretching the cuticle. Bar = 2 μm.

Nepi et al. (1996) hypothesized that the thin cuticle of *Cucurbita pepo* nectaries contains very little wax because it is not stained by auramine O, a specific dye for this substance (see Table 2). Nectar may possibly exude through a permeable cuticle. Cuticle permeability to secretory products has been also postulated in nectaries of the orchid *Maxillaria coccinea* (Stpiczyńska et al., 2003).

In some species of the genus *Euphorbia* the cyathial nectary is covered by a cuticle that is not uniform in thickness, being thinner in the "secretory pits" through which the nectar exudes (Arumugasamy et al., 1990a). Kronestedt et al. (1986) reported pores in the cuticle above the nectar secreting trichomes of *Abutilon* sp. (Malvaceae).

The cuticle may have microchannels from which the nectar exudes (Davis et al., 1988; Stpiczyńska, 2003). In *Platanthera chlorantha* (Orchidaceae), the microchannels appear as fibrillar outgrowths of the outer epidermal cell wall (Fig. 3), as also observed in *Abutilon* sp. (Kronestedt et al., 1986). In *Helleborus foetidus* (Ranunculaceae), microchannels are narrow

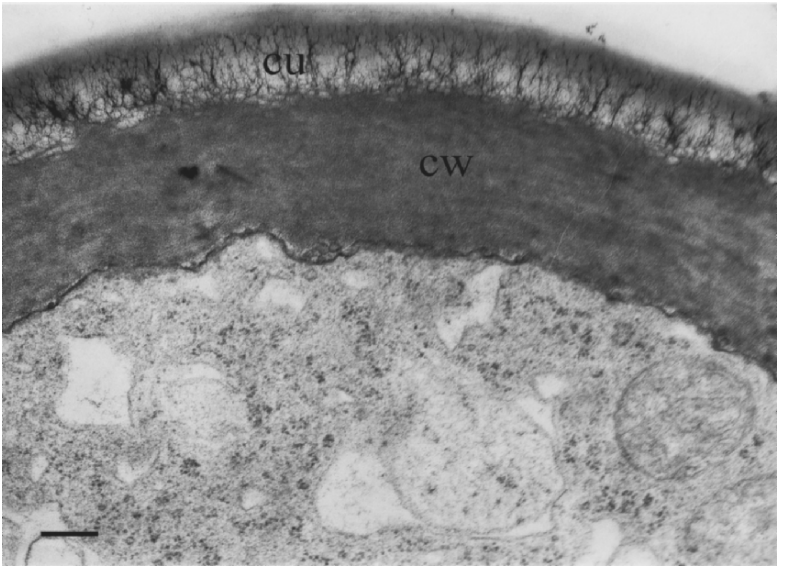

Figure 3. Epidermal cell wall and cuticle of a secretory hair of the *Platanthera chlorantha* (Orchidaceae) floral nectary. Nectar presumably flows out through fibrillar outgrowths of the outer cell wall (microchannels) present in the cuticle. (This picture was kindly provided by Malgorzata Stpiczyńska, Department of Botany, Agricultural University in Lublin, Poland.) Bar = 0.4 μm; cu = cuticle; cw = cell wall.

tubular interruptions of the cuticle in continuity with the cell wall; some of them seem to have direct communication with the outside (Koteyeva, 2005). Very similar microchannels are described in the cuticle of epidermal cells of the *Echinacea purpurea* (Asteraceae) nectary, although they have no direct communication with the outside (Wist & Davis, 2006).

Complex cuticle organization with a lamellar-type outer layer and a reticulate-type inner one has been described in the floral nectary of *Aptenia cordifolia* (Aizoaceae) and *Limodorum abortivum* (Orchidaceae) (Meyberg & Kristen, 1981; Figueiredo & Pais, 1992).

2.1.1 Secretory trichomes

The nectary epidermis may have trichomes as the secretory structures. The morphology of trichomes varies, and includes the following types:

- Unicellular trichomes as in the floral nectaries of *Lonicera* (Caprifoliaceae) (Fahn & Rachmilevitz, 1970)

- Multicellular, linear trichomes as in the floral nectaries of *Abutilon* (Kronestedt-Robards et al. 1986) and *Hibiscus rosa-sinensis* (Malvaceae) (Sawidis et al., 1987a)
- Multicellular, capitate trichomes as in the extrafloral nectaries of *Vicia faba* (Davis et al., 1988) (Table 1)

The detailed ultrastructural development of nectary trichomes has been investigated in *Abutilon* (Kronestedt-Robards et al., 1986) and in *Hibiscus* (Sawidis et al., 1987a). The first event to take place is an outgrowth of epidermal cells followed by periclinal division. Volume increase of epidermal cells is accompanied by cell polarization, manifested by displacement of organelles towards the apical region.

The most specialized cells of pluricellular trichomes are the basal, stalk, and tip cells. The basal cells (situated at the level of the other epidermal cells) have a greater number of plasmodesmata than adjacent cells (Sawidis et al., 1987b). After entering the secreting hairs, pre-nectar flows from cell to cell through plasmodesmata (symplastic route) reaching the tip cell (Sawidis et al., 1987b). The apoplastic route of pre-nectar is impeded by lignification or complete cutinization of the lateral walls of the stalk cells (Fahn, 1979b; Sawidis et al., 1987a; Davis et al., 1988). The tip cells have very elaborate systems of ER, dictyosomes, and vesicles and they are thought to be involved in granulocrine secretion (Fahn, 1979b; Kronestedt-Robards et al., 1986; Sawidis et al., 1987b, 1989).

The floral nectary of *Tropaeolum majus* (Tropaeolaceae) has epidermal hairs, but the main source of nectar is the nectary parenchyma and nectar is exuded through the modified stomata (Rachmilevitz & Fahn, 1975).

2.1.2 Nectary-modified stomata

Nectar exudation through stomata appears to be the most common manner of nectar release (Table 1, Bernardello, 2007). Nectar flow may be so high that the stomatal aperture enlarged (Fig. 4). The nectary stomata may be located on the surface of the nectary or in deep depressions (Fig. 5)

Stomata involved in nectar secretion have been described as “nectarostomata” (Smets & Cresens, 1988). They are considered to be “modified” with respect to leaf stomata because they are not able to finely regulate their aperture (Davis & Gunning, 1992, 1993). In actively secreting nectaries, the stomata are raised slightly above the epidermis, while most stomata of not yet secreting nectaries are open but not raised (Gaffal et al., 1998; Nepi

et al., 1996). After measuring the volume flux of the floral nectar of *Digitalis purpurea* (Scrophulariaceae) through individual stomatal apertures, Gaffal et al. (1998) concluded that only a fraction of the total number of stomata per nectary would be sufficient to release the amount of nectar produced. In *Hedera helix* (Araliaceae; Vezza et al., 2006) and *Echinacea purpurea* (Asteraceae; Wist & Davis, 2006) closed immature stomata were present on the surface of the nectary during the secretion phase.

The stomatal apertures are continuous with intercellular spaces of the nectary parenchyma (Gaffal et al., 1998) and there is evidence to suggest that modified stomata are unable to closely regulate nectar flow through them (Davis & Gunning, 1993; Razem & Davis 1999). For instance, asynchrony in stomatal development (pores wide open a few days before the start of nectar secretion and after secretion has ceased) suggests little coordination between pore opening and nectar release (Davis & Gunning, 1992; Davis, 1997; Razem & Davis, 1999). According to Teuber et al. (1980) and Davis and Gunning (1993), leaf and nectary stomata differ in their response to various stimuli. Nectary stomata remained open under all treatment conditions, suggesting that nectary stomata lack the turgor- and ion-mediated movements generally found in leaf stomata.

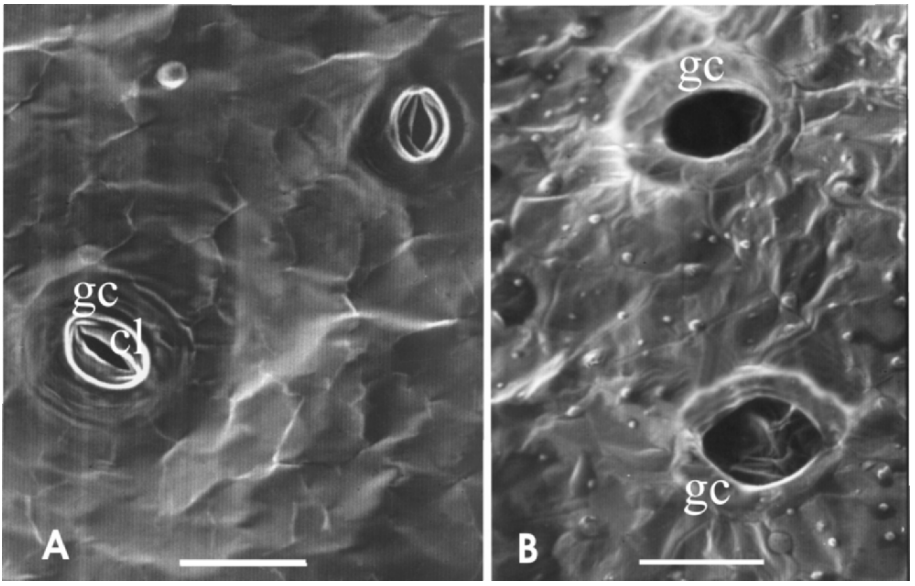

Figure 4. Nectary stomata of *Cucurbita pepo* male flower before (*left*) and after (*right*) nectar secretion. The inner portion of guard cells (gc), where the outer cuticular ledge (cl) is evident before nectar secretion (A), is collapsed at the end of secretion (B). Bar = 50 μm.

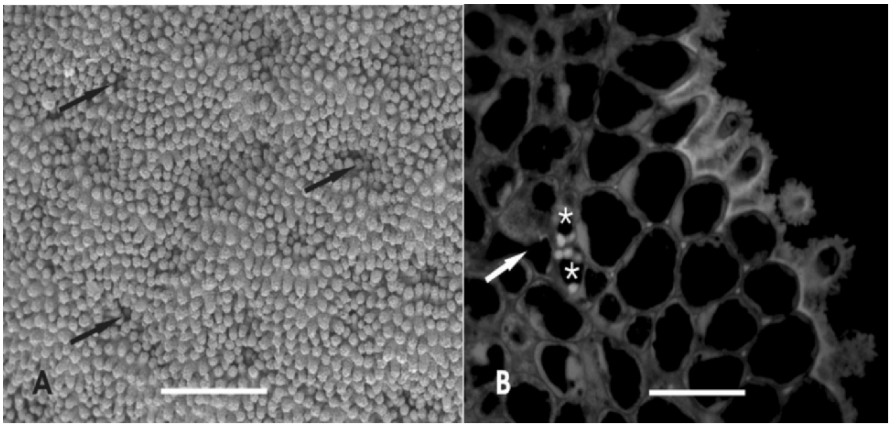

Figure 5. The nectary of *Fatsia japonica* (Araliaceae) A. Electron micrograph (SEM) of the nectary surface shows numerous hollows (*arrows*) that indicate the position of the stomata. Bar = 100 µm. B. Oblique section of the nectary stained with PAS and auramine O and observed by epifluorescence. A thick cuticle with a complex reticulate pattern can be observed. The guard cells of the stoma (*asterisks*), easily recognizable by their small size and starch content, are located at the level of the inner epidermal layer. The arrow indicates the substomatal chamber. Bar = 20 µm.

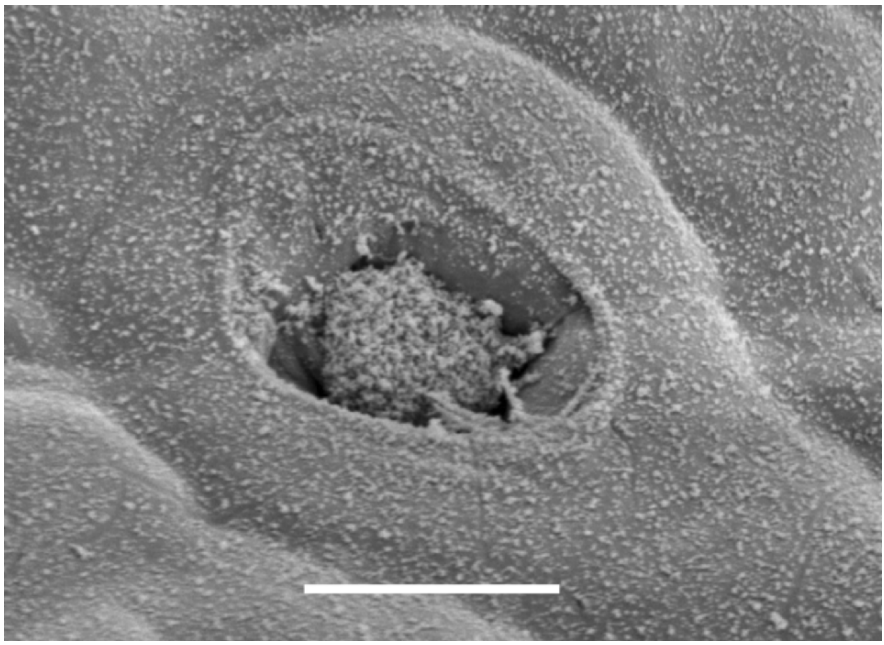

Figure 6. A nectarostoma of *Daphne sericea* (Thymelaeaceae) occluded by granular material. Bar = 10 µm.

Instead of pore closure by guard-cell movements, closure of the modified stomata of the floral nectary may occur exclusively by occlusion in some species (Fig. 6). The occluding material is of uncertain nature (Gaffal et al., 1998). It cannot be excluded that nectar may crystallize in the stomatal aperture. It has been hypothesized that occlusion of the stomatal pores may be a mechanism to seal off potential entry sites for pathogens (Davis, 1997; Razem & Davis, 1999). Micro-organisms have been found in the stomatal apertures (Gaffal et al., 1998) and the nectary has been recognized as the primary site of infection by *Erwinia amylovora*, the agent of fire blight disease in *Malus* and *Pyrus* (Buban et al., 2003).

2.2 Nectary parenchyma

The nectary parenchyma is generally composed of a few to several layers of small, isodiametric cells, generally with thin walls, dense granular cytoplasm, small vacuoles, and relatively large nuclei. Even if there are different types of nectaries and they have a non-uniform structure they always belong to the class of secreting cells. Owing to their secretory activity, all these kind of cells have extra copies of DNA realized by means of multinucleate cells, polyploid nuclei, or polytenic chromosomes (D'Amato, 1984). Nevertheless, even in ultrastructural studies little attention has been paid to the nuclei of nectary secreting cells and multinucleate cells were never observed.

These peculiar cytological characteristics mean that the nectary parenchyma can very often be distinguished easily from the ground parenchyma. Unusually collenchymatous cells with thick walls were observed in the nectary of *Maxillaria coccinea* (Orchidaceae) (Stpiczyńska et al., 2003).

Vacuole size in nectary parenchyma cells varies according to the stage of nectary development: small vacuoles are present in the pre-secretory phase, and may increase in volume at the time of secretion, but generally a sharp increase in vacuole volume takes place after secretion. The cytoplasm is usually rich in ribosomes and mitochondria. These organelles generally increase in number at the moment of secretion, indicating increased energy requirements for nectar production. Intercellular spaces are present and increase at the time of secretion.

It is not uncommon to find cells undergoing cell division in actively secreting nectaries (Gaffal et al., 1998; Nepi et al., 1996). Continued cell division and the lack of subsequent cell extension in small-celled nectariferous tissue are more or less comparable to meristematic tissue (Gaffal et al., 1998 and references therein). This implies that nectary parenchyma cells

maintain the potential of cell regeneration, at least in some species such as *Digitalis purpurea* (Gaffal et al., 1998), *Cucurbita pepo* (Nepi et al., 1996), and *Helleborus* sp. (Vesprini et al., 1999).

The structure and ultrastructure of nectary parenchyma appears to depend mainly on two features: the mechanism of pre-nectar transport (through the apoplast or symplast) and the source of nectar carbohydrates (starch reserves or direct photosynthesis). The term pre-nectar refers to substances transported into nectary tissue to be transformed into nectar by the nectary parenchyma or epidermal cells.

On the basis of numerous plasmodesmata between the cells, Fahn (1979b) proposed the symplast as the main path of pre-nectar transport into the parenchyma cells of *Lonicera japonica*, but evidence is also available for pre-nectar transport via the apoplast (Davis et al., 1988; Peng et al., 2004). The two mechanisms may possibly take place simultaneously (Wergin et al., 1975; Davis et al., 1986; Davis et al., 1988; Stpiczyńska, 1995; Stpiczyńska et al., 2003; Wist & Davis, 2006). Plasmodesmata are generally found between nectary parenchyma and subnectary parenchyma cells; their fine structure in nectaries has been reviewed by Eleftheriou (1990).

Nectar secretion, i.e., the transfer of nectar outside the protoplast of parenchyma cells, may be granulocrine or eccrine. Eccrine secretion involves transport of individual molecules across the secretory cell membrane. In granulocrine secretion molecules are grouped and transported in ER- or dictyosome-derived vesicles that fuse with the plasmalemma and release the molecules outside the nectary cells (Fahn, 1988). When granulocrine secretion occurs, parenchyma cells are rich in ER cisternae, dictyosomes, and vesicles (Rachmilevitz & Fahn, 1973; Fahn, 1987b; Arumugasamy et al., 1990b) and an increase in the number of these organelles indicates imminent nectar secretion. Robards and Stark (1988) demonstrated an extensive "secretory reticulum", i.e., an internal membrane system closely associated with the plasmalemma, within the secretory trichomes in the nectary of *Abutilon*.

On the other hand, when ER cisternae and Golgi vescicles are rare and their number remains almost unchanged during flower development, eccrine secretion is likely (Elias et al., 1975; Eriksson 1977; Nepi et al., 1996; Razem & Davis, 1999; Stpiczyńska et al., 2003).

Different pre-nectar transport mechanisms have been documented in the same family (Eriksson, 1977; Davis et al., 1988; Nepi et al., 1996; Peng et al., 2004), in flowers of the same species (Meyberg & Kristen, 1981) and

are presumably possible in a single nectary (Schnepf & Pross, 1980; Pate et al., 1985).

The source of nectar carbohydrates may be immediate photosynthesis by the nectary itself or by any other part of the plant, or may require temporary starch storage in the parenchyma cells (Pacini et al., 2003). The two modes are strictly related to the rate of secretion: a very high nectar secretion rate requires starch storage in the parenchyma with big amyloplasts differentiating before secretion (Durkee et al., 1981; Belmonte et al., 1994; Nepi et al., 1996; Maldonado & Otegui, 1997), whereas a low rate of nectar secretion is often associated with chloro-amyloplasts with poor thylakoid structure, irregular shape and plastoglobuli (Stpiczyńska, 1997, 2003; Razem & Davis, 1999). Floral nectaries may manifest both modes of carbohydrate supply, whereas in extrafloral ones nectar is always derived from direct photosynthesis.

In plants with a high nectar secretion rate and starch-storing nectary parenchyma, there is a dramatic increase in the number of mitochondria just prior to anthesis, indicating that the comparatively rapid breakdown of stored starch requires more immediate energy than the gradual storage of starch during flower bud development (Durkee et al., 1981).

The source of nectar carbohydrates and the manner of nectar secretion seem correlated: *Passiflora*, *Cucurbita*, and *Rosmarinus* have eccrine secretion and contain a lot of starch; other species with little or no starch at all in the nectary may have granulocrine or eccrine nectar secretion (O'Brien et al., 1996; Nepi et al., 1996) (Table 1).

Although some nectaries are green, presumably due to chlorophyll in their plastids, it seems unlikely that the nectary parenchyma plastids themselves produce the starch grains observed inside them. Nectaries are often concealed and only receive very diffuse light. This may be why the thylakoids and grana are underdeveloped. When nectaries are exposed directly to the light, photosynthesis in the nectary parenchyma cannot be excluded *a priori*. The main photosynthetic activity probably takes place in the subnectary parenchyma where a greater quantity of chlorophyll is located (Fig. 7).

The vacuoles of the nectary parenchyma or subnectary parenchyma cells may contain different types of inclusions. Calcium oxalate crystals in the form of druses or raphides have often been found in floral and extrafloral nectaries (Davis et al., 1988; Horner et al., 2003; Stpiczyńska et al., 2003). It has been demonstrated that Ca^{2+} inhibits plasma membrane ATPase (Leonard

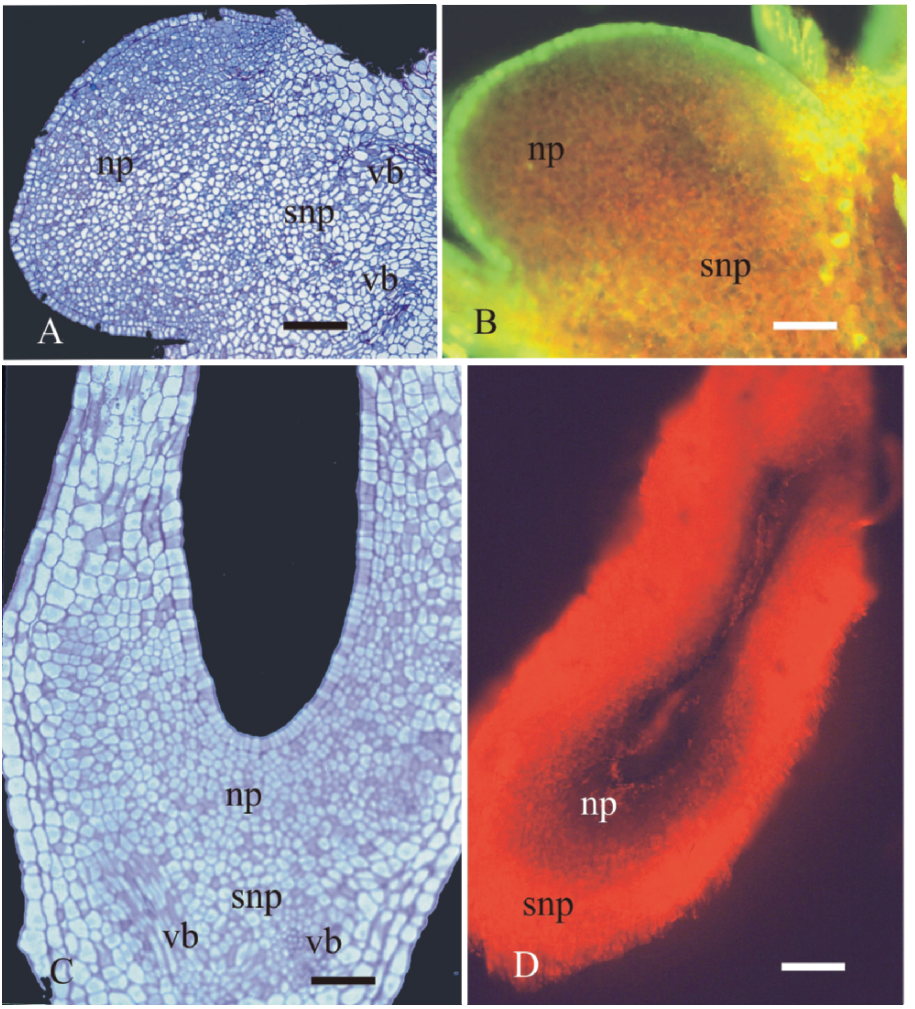

Figure 7. Floral nectary of *Linaria vulgaris* (A, B) and *Helleborus foetidus* (C, D), semithin-sections under bright light (A, C stained with PAS and TBO, see Table 2) and hand sections under UV light (B, D). In both species the main fluorescence of chlorophyll (i.e., the presence of chloroplasts) is located in the subnectary parenchyma where the main branch of vascular bundles is present. np = nectary parenchyma; snp = subnectary parenchyma; vb = vascular bundles. A, B, D bar = 400 µm, C bar = 150 µm.

& Hodges, 1980) and the mechanism of sucrose transport in plants is also known to involve ATPase (Giaquinta, 1979), thus druses and raphides may immobilize calcium in the nectary where active sugar transport is presumably occurring. Another putative function of calcium oxalate crystals in the parenchyma cells of *Glycine max* floral nectaries was descibed by Horner et al. (2003): these crystals sequester calcium during nectary development,

causing the formation of very thin cell walls in the nectary parenchyma. It has, however, also been suggested that they may simply be excretory products and discourage herbivory by invertebrates (Davies, 1999).

Protein bodies, finely granular and irregular in shape, have been found in the vacuoles of *Maxillaria coccinea* (Orchidaceae) (Stpiczyńska et al., 2003) and very similar structures in the floral nectary of *Passiflora* (Durkee et al., 1981; Durkee, 1982). Their role is unclear and requires further investigation. They cannot be precursors of the protein component of nectar because of their presence in secreting cells.

The floral nectary parenchyma and epidermal cells of most plants remain intact throughout secretion (merocrine secretion). In a few cases, secretion of nectar implies cell death (holocrine secretion) as reported for the floral nectaries of *Turnera ulmifolia* (Elias et al., 1975), *Helleborus foetidus* and *H. bocconei* (Vesprini et al., 1999), and *Glycine max* (Horner et al., 2003). A widespread degenerative process occurs in *T. ulmifolia* and *G. max*, but only cell-by-cell in *Helleborus*, probably involving spatial reorganization of secreting cells (Vesprini et al., 1999). This different pattern of cell degeneration is probably related to the very different duration of nectar secretion: short in *Turnera ulmifolia* and *Glycine max* (a few hours and 24 h, respectively) and long in *Helleborus* (about 20 days). The long duration of nectar secretion in *Helleborus* is not compatible with a rapid and massive degeneration of secreting cells.

Horner et al. (2003) reported that in *Glycine max*, before the nectary parenchyma and epidermal cells undergo programmed cell death, they produce compounds of unidentified chemical composition that engorge their central vacuole which has an apparently “discontinuous tonoplast”.

The fate of the nectary parenchyma after secretion may have different patterns when nectar secretion does not cause cell death. The nectary tissue may

- Be involved in nectar reabsorption (Nepi et al., 1996)
- Differentiate into another tissue (parenchyma tissue, as in the case of septal nectaries of certain monocots; see “Gynopleural (septal) nectaries” on page 154)
- Degenerate (Fig. 8)

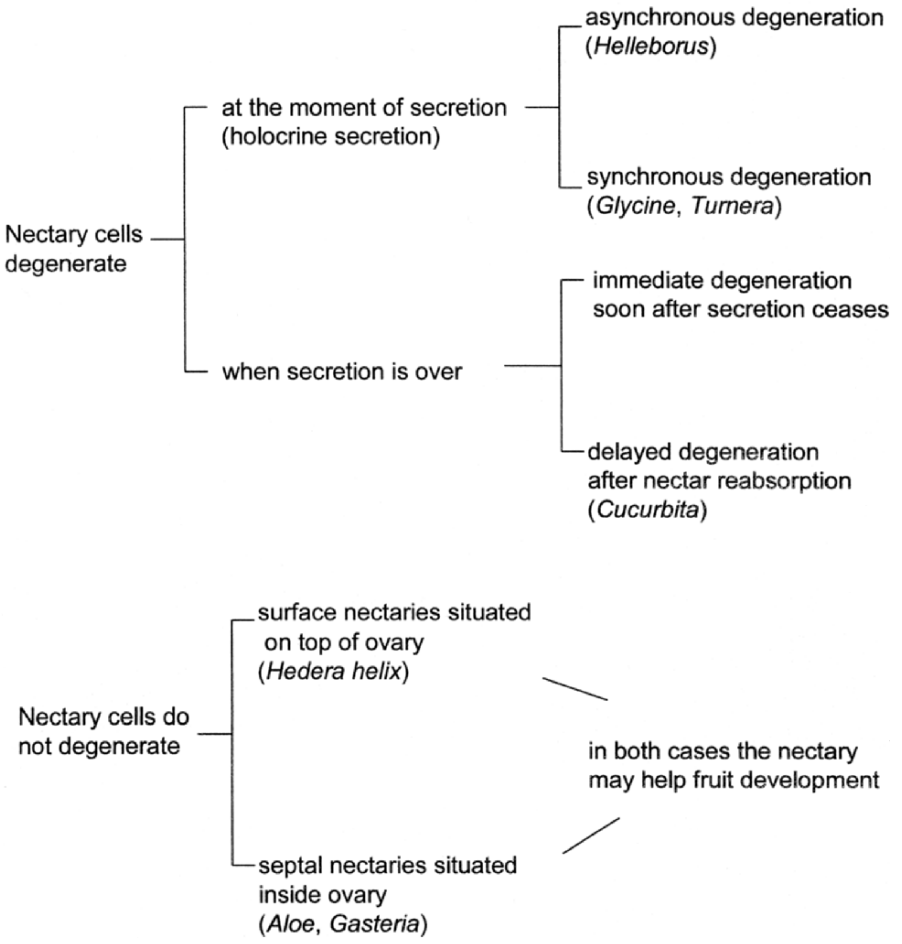

Figure 8. Nectary fates and cell degeneration after nectar secretion.

Several cases of cell degeneration have been reported in nectaries after secretion. Typical evidence of programmed cell death (PCD) such as nuclear disorganization, cytoplasmic condensation, and disruption of ER cisternae was observed in *Arabidopsis thaliana* (Zhu & Hu, 2002). A continuous increase in vacuole volume in post-secretory nectaries is often associated with autophagic events, revealed by the presence of amyloplasts and other organelles in the vacuole (Rachmilevitz & Fahn, 1973; Cecchi Fiordi & Palandri, 1982; Kronestedt et al., 1986; Belmonte et al., 1994; O'Brien et al., 1996). However, examples of nectary tissue degeneration were not reported in a recent review on PCD in floral organs (Rogers, 2006).

In *Rosmarinus officinalis,* the vacuole volume increases after secretion, the cytoplasm darkens and its volume decreases. There is a distinct increase in ER cisternae, which appears to be related to the lytic process of the disintegrating protoplast (Zer & Fahn, 1992). Similar processes of nectary degeneration after secretion were observed in septal nectaries of banana flowers (Fahn & Benouaiche, 1979) and in extrafloral nectaries of *Sambucus nigra* (Fahn, 1987). Multilamellar bodies characterized by membranous conglomeration have been associated with degradative processes in a number of species (Davis et al., 1986 and references therein; Nepi et al., 1996).

According to Durkee et al. (1981), a complete breakdown of the secretory tissue occurs in the floral nectary of *Passiflora* in the post-secretory phase. Intercellular spaces enlarge considerably and cell walls become compressed and collapsed. The cytoplasm becomes electron-translucent and the internal membranes of plastids and mitochondria show signs of considerable disorganization. Collapsed and compressed cells were also observed in the epidermis of *Hexisea imbricata* (Orchidaceae) (Stpiczyńska et al., 2005a).

2.2.1 Patterns of plastid development in nectary parenchyma cells

Plant cell differentiation is a process in which almost all cell compartments are involved, among which plastids always play a crucial role. Proplastids of meristematic cells differentiate into other types of plastids. In vegetative organs, such as leaves, plastid differentiation is commonly unidirectional, while in flower cells plastids may interconvert and dedifferentiation is more frequent than in other plant parts (Pacini et al., 1992; Clement & Pacini, 2001).

Proplastids are the “meristematic” plastid type always encountered in all the young stages of nectaries studied ultrastructurally (Nepi et al., 1996). Generally proplastids undergo some divisions before beginning to differentiate (Pacini et al., 1992; Nepi et al., 1996).

Plastid differentiation may follow different pathways according to the species and the stage of nectar development. The features of plastids in adult parenchyma nectary cells vary widely at the moment of nectar secretion, because of the different development of thylakoids and grana and the different degrees of starch storage (Fig. 9). Undifferentiated plastids (proplastids) are present in the very early stages of nectary development. Close to flower anthesis, chloro-amyloplasts may differentiate and are generally present in nectary parenchyma when secretion begins (Figs. 9 and 10). They contain very few small starch grains per plastid. In some cases, chloro-amyloplasts

lose their thylakoid structure and starch grains increase in size a few days before anthesis (Zer & Fahn, 1992; Fahn & Shimony, 2001). In other cases, proplastids differentiate into amyloplasts and store great amounts of starch in many large grains per plastid before nectar secretion begins (Durkee et al., 1981; Figueiredo & Pais, 1992; Pais & Figueiredo, 1994; Nepi et al., 1996) (Figs. 9 and 10). In *Passiflora biflora, Rosmarinus officinalis*, and *Cucurbita pepo* (Durkee et al., 1981; Zer & Fahn, 1992; Nepi et al., 1996), nectary parenchyma proplastids start to accumulate starch derived from the photosynthesis of other floral parts during pre-anthesis (Pacini et al., 2003 and references therein). In these three species, starch also accumulates in the epidermis, though the number of grains per plastid is fewer than in parenchyma cells. Amyloplasts in the nectar-producing parenchyma are generally almost devoid of stroma and packed with starch (Fig. 10) (Nepi et al., 1996). They also contain many starch grains per amyloplast; this increases starch surface area, facilitating and speeding hydrolysis during nectar production.

The type of plastids and presence of starch are heterogeneous features of orchid floral nectaries (Table 1 and Fig. 9). Plastids may have an undifferentiated appearance and contain osmiophilic bodies (*Gymnadenia conopsea* and *Platanthera chlorantha)*; they may have thylakoid-like membranes that resemble choloroplasts (*Hexisea imbricata, Maxillaria coccinea, Platanthera bifolia),* or they may be amyloplasts *(Limodorum abortivum).*

Undifferentiated plastids and chloroplasts may or may not store starch in the pre-secretory phase. No starch grains were observed in plastids of the nectary cells of the orchids *Gymnadenia conopsea* and *Maxillaria coccinea* (Table 1 and Fig. 9), however starch has been found in plastids of other orchids, such as *Hexisea imbricata, Platanthera bifolia,* and *Platanthera chlorantha.*

The quantity of starch in plastids peaks in mature buds and decreases with the onset of nectar production. Thus many authors infer that hydrolysis of starch in the parenchyma contributes directly to nectar carbohydrate content (Rachmilevitz & Fahn, 1973; Durkee et al., 1981; Zer & Fahn, 1992; Nepi et al., 1996; Pacini et al., 2003; Peng et al., 2004). The sugars derived by starch breakdown can also be used to produce energy for the process of secretion. The general pattern of starch decreasing at the moment of nectar secretion was not found in *Trifolium pratense* (Fabaceae) and *Ecballium elaterium* (Cucurbitaceae). In red clover, starch grains in plastids were actually more numerous and larger in florets at the end of nectar production (Eriksson, 1977). In *Ecballium elaterium,* plastids have well-differentiated thylakoids and grana in the early stage of nectary development; they store starch, reaching

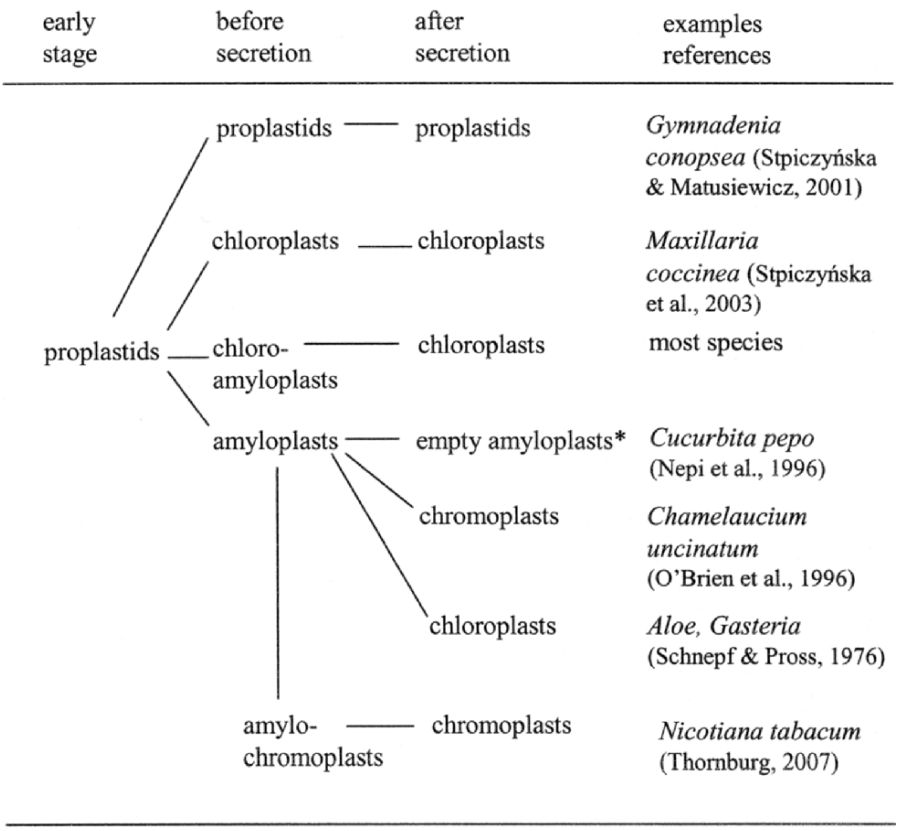

Figure 9. Plastid differentiation pathways during nectary development. In *Gymnadenia conopsea*, proplastids do not differentiate and are probably not so much involved in nectar production. Amyloplasts present a wide range of differentiation. Before nectar secretion they may become amylochromoplasts, after nectar secretion and starch hydrolysis they may remain empty amyloplasts or differentiate into chloroplasts or chromoplasts.

*Empty amyloplasts can be involved in a temporary storage of reabsorbed carbohydrates if nectar was not totally consumed by flower visitors.

peak accumulation in mature buds, but there is apparently no hydrolysis at the time of nectar secretion, probably because of the very small amount of nectar secreted (Fahn & Shimony, 2001).

The amyloplast membrane remains integral during starch hydrolysis in *Passiflora* sp. and *Cucurbita pepo* (Durkee et al., 1981; Nepi et al., 1996). Plastid degeneration generally only occurs after complete starch hydrolysis and/or nectar resorption. The degeneration of the nectary with empty

amyloplasts at the end of secretion or after nectar reabsorption seems to be a general feature.

In *Aloe* and *Gasteria*, which have septal nectaries, dedifferentiation of amyloplasts to chloroplasts has been recorded after nectar secretion (Schnepf & Pross, 1976; Nepi et al., 2005). This dedifferentiation allows secreting cells to transform into fruit parenchyma cells.

Other patterns of nectary plastid development have also been observed. In *Chamelaucium uncinatum* (Myrtaceae), the nectary parenchyma cells have chloroplasts and secrete nectar for 11 days. At the end of the secretion period the nectary becomes red, probably because of transformation of chloroplasts into chromoplasts (O'Brien et al., 1996). The pattern of nectary plastid development is more complicated in *Nicotiana tabacum*, where the nectary parenchyma cells differentiate into chloroplasts in the early stages. Later they accumulate starch, becoming amyloplasts, and when starch is hydrolysed they accumulate β-carotene becoming amylochromoplasts (Fig. 9) (Thornburg, 2007).

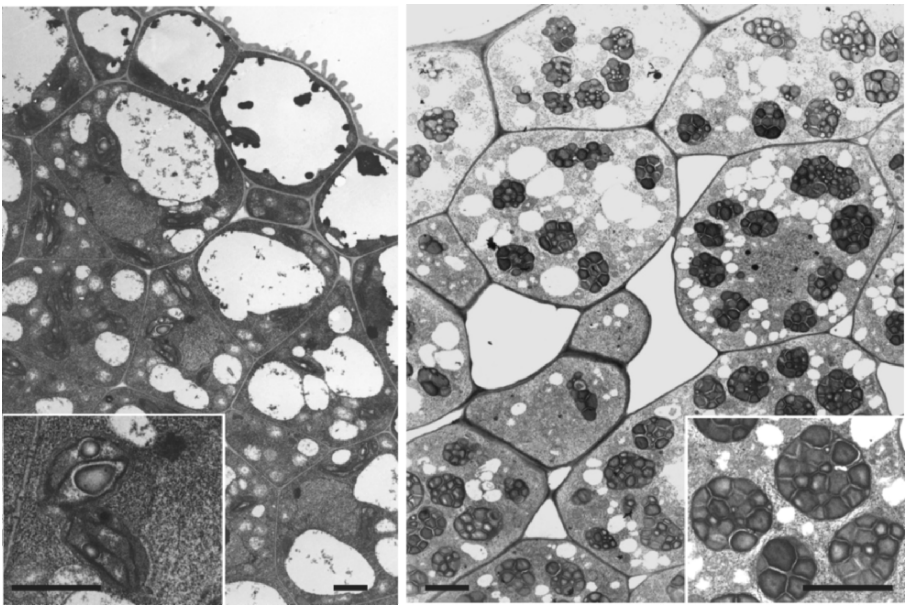

Figure 10. Floral nectaries of *Arabidopsis thaliana* (*left*) and *Cucurbita pepo* (*right*). Inserts show the details of plastid structure in the nectary parenchyma cells. In *A. thaliana* there are chloroamyloplasts with few thylakoids and very few starch grains. In *C. pepo* there are very large amyloplasts with many large starch grains. Bars = 5 μm.

2.3 Subnectary parenchyma

The subnectary parenchyma is located below the nectary parenchyma, from which it is generally easily distinguished because it consists of larger cells, with bigger vacuoles, less dense cytoplasm, and larger intercellular spaces. Durkee (1982) reported plasmodesmata between nectary and subnectary parenchyma cells in the extrafloral nectary of *Passiflora*, suggesting that these tissues cooperate in the secretion of nectar. Generally neither, the ER nor Golgi apparatuses in the subnectary parenchyma cells show the unusual degree of development and swelling found in the nectary parenchyma cells (Durkee, 1983). Insignificant ultrastructural changes take place in subnectary parenchyma cells approaching secretion, and generally the vacuole increases in size at secretion.

As described earlier, subnectary parenchyma is generally richer in chloroplasts than nectary parenchyma. Vascular bundles are always present in subnectary parenchyma. In most cases the xylem vessels stop in this tissue while phloem strands branch into the nectary parenchyma (Fig. 11).

Oil and mucilage cells were described by Sawidis (1998) in the subnectary parenchyma of *Hibiscus rosa-sinensis*. Because of the water-binding capacity of mucilage, with rapid water uptake and slow release, it was hypothesized that in this species mucilage cells offer an ideal regulation mechanism for water balance during nectar secretion and efficient protection of nectary tissue against water stress damage. Oil cells, on the other hand, are supposed to be involved in nectary protection against herbivores.

2.4 Nectary vasculature

The vasculature brings raw materials for nectar production to the nectary. Frey-Wyssling and Agthe (1950) suggested a correlation between the vascular supply of the nectary and the concentration of nectar. Nectaries that secrete very concentrated nectar are vascularized by phloem only. Nectaries secreting nectar with low sugar concentrations are vascularized equally by phloem and xylem or primarily by xylem. This hypothesis was supported by observations in *Gossypium* (Wergin et al., 1975), *Abutilon* (Gunning & Hughes, 1976), and *Hibiscus* (Sawidis et al., 1987a), but was not always confirmed in subsequent studies (Dafni et al., 1988; Zer & Fahn, 1992).

Although some nectaries are reported to be vascularized by xylem and phloem, the last branches reaching the nectary parenchyma are generally phloem elements, which may reach the area of the epidermis. This feature is

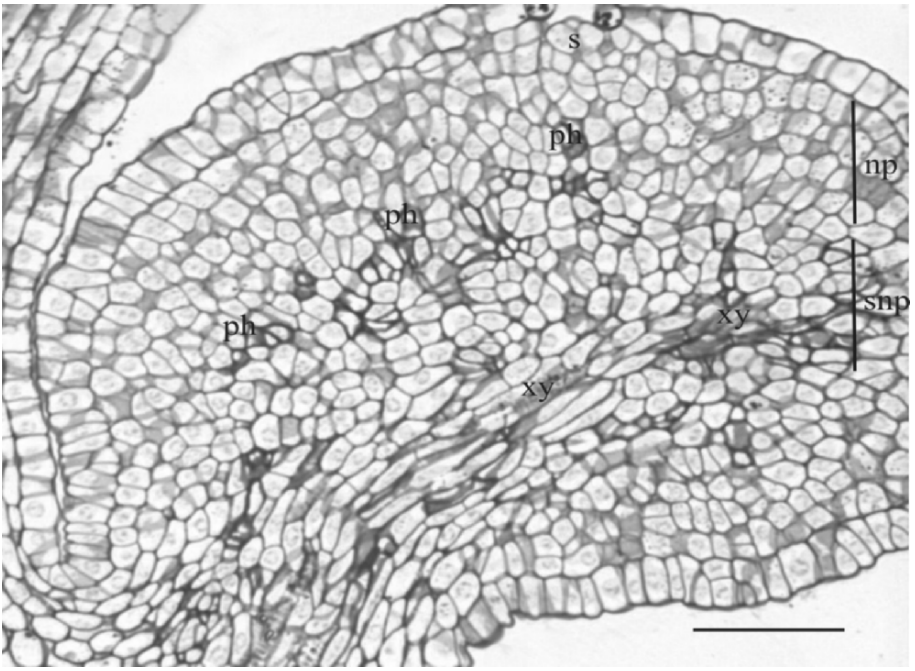

Figure 11. The floral nectary of *Daphne sericea* (Thymelaeaceae). Xylem vessels (xy) stop in the subnectary parenchyma (snp) while phloem strands branch into the base of the nectary parenchyma (np). s = stoma. Bar = 80 µm, stained with PAS.

often encountered in nectaries of Asteraceae (Wist & Davis, 2006). Phloem alone supplies the floral nectaries of most species of Brassicaceae and a direct relation has been demonstrated between the abundance of phloem supply and nectar carbohydrate production (Davis et al., 1998).

Well-developed wall ingrowths, reminiscent of those of transfer cells (Pate & Gunning, 1972), have been detected in the companion cells and are common in nectary phloem (Davis et al., 1988; Belmonte et al., 1994; Razem & Davis, 1999; Wist & Davis, 2006). The increased surface area of the companion cell membrane around these ingrowths is thought to enhance unloading of pre-nectar components from sieve tube elements and their direct transfer to adjacent phloem parenchyma and intercellular spaces (Davis et al., 1988; Razem & Davis, 1999; Wist & Davis, 2006). Unusually large companion cells, characterized by large membrane-bound protein bodies, were reported in the floral and extrafloral nectaries of different species of *Passiflora* (Durkee et al., 1981; Durkee, 1982). In these “intermediary cells”, wall ingrowths are not evident, but the unloading process may be favoured by their large surface area. The function of the membrane-bound protein bodies is obscure.

3 GYNOPLEURAL (SEPTAL) NECTARIES

Fahn (1979a) formulated a topographical classification of floral nectaries, differentiating nine different types. Among them, the "ovarial nectary" type includes nectaries that are situated in the septal region between adjacent carpels, known as septal or, more recently, gynopleural nectaries (Smets & Cresens, 1988). Gynopleural nectaries are largely absent in dicotyledons, although there are non-secretory septal slits in *Saruma* (Endress, 1994), *Cneorum tricoccum*, *Koelreuteria paniculata*, *Ruta bracteosa* and a few other dicotyledons (Schmid, 1985). On the other hand, they are the most common type of floral nectary in monocotyledons (Smets et al., 2000, Table 1) and are therefore considered separately from the other types of floral nectaries. According to Rudall (2002), septal nectaries have been lost several times in monocot evolution, probably in association with the emergence of different pollination syndromes.

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 (secondary presentation). Nectar must therefore flow through auxiliary ducts—up to 13 cm long in *Milla biflora*—to reach its site of emission (Vogel, 1998b; Bernardello, 2007; Pacini & Nepi, 2007). The morphological characters of gynopleural nectaries were reviewed from a systematic point of view by Daumann (1970) and Smets et al. (2000) and from a functional perspective by Schmid (1985). A very thin and sometimes apparently discontinuous cuticle is present on the surface of epithelial cells (Fahn & Benouaiche, 1979; Nepi et al., 2005). The nectar cavity is lined by a layer of secretory epithelial cells that may overlie a subsidiary glandular tissue, characterized by smaller cells with denser cytoplasm than the ground parenchyma cells, thus resembling the nectary parenchyma of floral nectaries. Wall ingrowths are very common in epithelial cells that, for this reason, are regarded as transfer cells. The differentiation of transfer cells in septal nectaries is supposed to be an anatomical device to increase nectar output via eccrine secretion (Schmid, 1985). Cell wall ingrowths are highly developed in *Aloe* and *Gasteria* (Schnepf & Pross, 1976; Nepi et al., 2005), but are not so abundant in the nectaries of banana and *Tillandsia* (Fahn & Benouaiche, 1979; Cecchi Fiordi & Palandri, 1982), where predominantly granulocrine secretion seems likely. Different extents of the subsidiary tissue were observed in different species of *Tillandsia* (Cecchi Fiordi & Palandri, 1982) and were related to nectar production rates.

The 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 male and female *Musa paradisiaca* flowers (Fahn & Kotler, 1972), where the cytoplasm became very electron-dense, plastids and mitochondria degenerated and the vacuole increased gradually in volume until it occupied most of the cell (Fahn & Benouaiche, 1979). On the other hand, transformation of nectary tissue into parenchyma, by elongation of epithelial cells and occlusion of the nectary cavity by acidic polysaccharides, has been reported in *Aloe, Gasteria,* and *Tillandsia* (Schnepf & Pross, 1976; Cecchi Fiordi & Palandri, 1982). Schnepf & Pross (1976) demonstrated differentiation of transfer cells in the epithelium of septal nectaries in some *Aloe* species. A short time before anthesis, they formed an elaborate system of wall protuberances along their outer walls. They redifferentiated in the developing fruit; losing the wall protuberances, increasing in size, and becoming parenchymatous cells. Rearrangement of these cells was accompanied by transformation of amyloplasts into chloroplasts, probably involved in photosynthesis to help fruit development.

A very complex type of secretion has been reported in several species with septal nectaries. A mixture of protein and polysaccharides was found in the septal nectaries of banana (Fahn & Benouaiche, 1979). Sajo et al. (2004) reported a ring of mucilage canals around the infralocular nectary of some Bromeliaceae. Poor nectar production and the presence of amorphous, hydrophilic, acid polysaccharides suggest that the nectariferous tissue may have a role in water and nutrient accumulation in *Tillandsia*, where nectaries are more developed in species growing in dry habitats (Cecchi Fiordi & Palandri, 1982).

4 EXTRAFLORAL NECTARIES

Extrafloral nectaries differ from floral nectaries in position and function. Extrafloral nectaries may be situated on virtually any vegetative structure, but most often on the upper half of the petiole or near the base of the leaf blade (Elias, 1983). They may be associated with floral structures: on the rachis of the inflorescence, near the base of flowers or their pedicel, on the calyx, or on the corolla. Regardless of position, extrafloral nectaries are never directly involved in pollination and their main function is to feed ants that protect the plant against herbivores (Beattie, 1985; Heil et al., 2001; Falcão et al., 2003; Ness, 2003; Vesprini et al., 2003). In some cases they are active during the flower bud stage (Anderson & Symon, 1985) or during fruit development (Vinoth & Yash, 1992; Morellato & Oliveira, 1994). It was recently demonstrated that the total number of extrafloral nectaries on a plant may be

affected by the intensity of herbivory (Wäckers et al., 2001; Mondor & Addicott, 2003) and that herbivore-induced plant volatiles are responsible for increased extrafloral nectar production (Choh et al., 2006; Kost & Heil, 2006).

According to Elias (1983), who modified the early classification of Zimmermann (1932), seven morphological types of extrafloral nectary can be observed: formless nectaries, flat nectaries, elevated nectaries, scale-like nectaries, hollow nectaries, pit nectaries, and embedded nectaries. They are usually small protuberances, which may be covered by protecting non-secretory hairs (Sousa e Paiva et al., 2001; Falcão et al., 2003). Different morphological types of extrafloral nectaries may co-occur in different positions even on the same leaf of a plant such as *Passiflora* sp. (Galetto & Bernardello, 1992; Blüthgen & Reifenrath, 2003). Two types of extrafloral nectaries—differing in morphology, anatomy, function, and nectar composition—were described in *Vigna unguiculata* by Pate et al. (1985). Four extrafloral nectary sites (petiole, calyx, corolla, fruit) can be recognized in *Campsis* (Bignoniaceae), which also has floral nectaries (Elias & Gelband, 1976).

As happens among floral nectaries, some extrafloral nectaries are also devoid of vascularization and lack the anatomical organization typical of nectaries. Elias (1983) described this type of nectary as non-vascularized, non-structural; examples are those located in the outer verticel of petals in certain Bromeliaceae, Zingiberaceae, Paeoniaceae, and Cactaceae (Galetto & Bernardello, 1992 and references therein). More frequently, extrafloral nectaries have a structure not very different from that of floral nectaries. The most frequent vascularization consists of phloem or phloem and xylem. A continuous thick cuticle covers the epidermal cells of the extrafloral nectaries and nectar release generally takes place through cuticle rupture. In some cases, such as in some Bromeliaceae species (Galetto & Bernardello, 1992) and *Solanum stramonifolium* (Solanaceae) (Falcão et al., 2003), nectar can be secreted through stomata. Secretory cells located under the epidermis may occur in one or several layers and are usually elongated and orientated along a vertical axis. Plastids in extrafloral nectaries are generally chloro-amyloplasts (Pacini et al., 2003, see also Table 1) with very few starch grains. In fact, extrafloral nectaries show less evident starch formation and degradation processes than floral nectaries (Durkee et al., 1981).

Extrafloral nectaries also generally have merocrine secretion, though holocrine secretion has been described for *Ailanthus glandulosa* (Clair-Maczulajtys & Bory, 1983). Holocrine secretion has also been reported in the

extrafloral nectaries of *Sambucus*, but the cells die and disintegrate after they have ceased to secrete nectar in the usual merocrine manner, thus cell disintegration in this species can be regarded as tissue degeneration after secretion (Fahn, 1987).

Compared to floral nectaries, a wider range of inclusions has been found in the vacuoles of the parenchyma cells in extrafloral nectaries:

- Dense osmiophilic material in *Euphorbia neriifolia* (Arumugasamy et al., 1990b).
- Tannins in *Euphorbia neriifolia* and *Ailanthus glandulosa* (Arumugasamy et al., 1990b; Clair-Maczulajtys & Bory, 1983).
- Calcium oxalate raphides or druses in *Turnera ulmifolia*, *Passiflora* sp. (Elias et al., 1975; Durkee, 1982; Elias, 1983).
- Anthocyanin in *Ricinus communis* (Baker et al., 1978).
- Crystalline protein bodies in *Ricinus communis*. Since they were not observed in very young nectaries, they are presumably associated with storage of retained nitrogen (Baker et al., 1978). There is no evidence that they are subsequently hydrolyzed; thus their participation in nectar production is unlikely.

In a recent survey in an Australian rainforest, Blüthgen & Reifenrath (2003) found 34 plant species bearing extrafloral nectaries. Plant organs with extrafloral nectaries were mostly leaves and leaf petioles. Both adaxial and abaxial positions were commonly involved.

5 NECTARY HISTOCHEMISTRY

Although not sufficient for a detailed study of nectary structure and function, light microscopy and histochemistry may provide a general view of the sites and organization of the various parts of nectaries.

Active floral nectaries may be located by staining inflorescences with neutral red. Nectary cells selectively accumulate this stain. However, this does not seem to work with extrafloral nectaries (Kearns & Inouye, 1993). Common histochemical techniques for the study of nectaries are listed in Table 2. Toluidine blue O is frequently used as general nectary stain. The periodic acid–Schiff (PAS) reaction is a simple informative staining technique that stains cell walls and starch in amyloplasts or, temporarily, in chloroplasts. It must be preceded by blockage of free aldehyde groups (e.g., with a saturated dimedone solution) to avoid artefacts (O'Brien & McCully, 1981).

Table 2. Histochemical techniques used for nectaries. bf = bright field; UV = ultraviolet light.

Specificity	Stain—optics	Cell components stained	Reference
General stains			
	toluidine blue O (TBO)—bf	cell walls, nucleus, cytoplasm	Beardsell et al., 1989; Link, 1991; O'Brien et al., 1996; Maldonado & Otegui, 1997; Stpiczyńska et al., 2005a
	acid fuchsin—bf	nucleus, cytoplasm	Maldonado & Otegui, 1997
Polysaccharides			
Total insoluble polysaccharides	PAS (periodic acid-Schiff)—bf	cell walls, cytoplasm, amyloplsts	Beardsell et al., 1989; Nepi et al., 1996; Maldonado & Otegui, 1997; Nepi et al., 2003
Starch	IKI (iodine-potassium-iodide)—bf	starch grains inside amyloplasts	Nepi et al., 1996; Stpiczyńska et al., 2003; Maldonado & Otegui, 1997
Acid polysaccharides	ruthenium red—bf	cell walls	Maldonado & Otegui, 1997; Fahn & Benouachie, 1979; Stpiczyńska et al., 2003; Stpiczyńska et al., 2005a
Lipids			
	sudan III—bf	cytoplasm, lipid droplets	Stpiczyńska et al., 2003
	sudan IV—bf	cytoplasm, lipid droplets	Davis et al., 1988; Fahn & Benouachie, 1979
	auramine O—UV	cuticle	Beardsell et al., 1989; Nepi et al., 1996; O'Brien et al., 1996; Nepi et al. 2003
Proteins			
	comassie brilliant blue—bf	cytoplasm, nucleus, vacuole	Maldonado & Otegui, 1997; Stpiczyńska et al., 2003; Stpiczyńska et al., 2005a
	bromophenol blue—bf	cytoplasm, nucleus, vacuole	Nepi et al., 1996;
Phenols			
	Millons reagents—bf	vacuole	Sawidis, 1998
Tannins			
	DMB (dimethoxy-benzaldehyde)—bf	vacuole	Sawidis, 1998

Bright-field and epifluorescence techniques can be useful to study nectary structure. Autofluorescence of chlorophyll can be used to highlight the distribution of chloroplasts in the nectary and subnectary parenchyma (see Fig. 5). Phenolic compounds, the lignin of xylem vessels, and cuticles can be located by autofluorescence. Details of the cuticle can be highlighted using Auramine O. When the fluorescence is strong enough, UV and visible light of an appropriate intensity can be used simultaneously. This makes it possible to observe samples treated with conventional stains and fluorochromes at the same time.

As far as electron microscopy is concerned, the zinc iodide–osmium tetroxide (ZIO) method is suitable for general impregnation of the endomembrane system of many plant, algal, and fungal tissues. It has also been used for staining subcellular compartments of the nectary (Machado & Gregorio, 2001), where it facilitated observation of membranes and helped to elucidate the role of nectary regions and cytoplasmic organelles in nectar secretion.

Conventional chemical fixation can damage cell components and any results from studies using this technique must be considered with caution, especially when applied to highly dynamic systems such as those operating during nectar secretion. To overcome such problems, the freeze-substitution technique was recently applied to the study of nectary ultrastructure and nectar secretion (Robards & Stark, 1988; Zhu et al., 1997; Zhu & Hu, 2002; Stpiczyńska et al., 2005b). According to Zhu et al. (1997), the membranes of organelles, vacuoles, and nuclei showed less shrinkage than with chemical fixation. With this technique, Robards and Stark (1988) observed an open extracytoplasmic space external to all the cells of the secretory hairs of *Abutilon*. According to these authors, the endomembrane system of nectar secretory cells is not appreciably affected by chemical fixation.

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